

THE TRANSPLANTATION OF AUERBACH'S PLEXUS IN THE SMALL INTESTINE OF A DOG TO THE BLADDER WALL AND OVARY, AND A NEURO-HISTOLOGICAL STUDY ON THE ATTITUDE OF THE TRANSPLANTED GANGLION CELLS IN THE PLEXUS

by

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I INTRODUCTION

The digestive tract is influenced by the autonomic nerve, namely, on principle it is promoted by the excitement of the parasympathetic nerve and inhibited by the sympathetic nerve. The intestine performing the peristaltic motion by mechanic stimulations without the extrinsic nerves is due to the action of intramural plexuses.

Since AUERBACH (1862) described the network structures of the nervous tissue in the muscle layer of the intestine, GREVING, DOGIEL, KUNTZ and others have investigated this plexus histologically and also, MAGNUS, ESVELD, YANASE and others have observed the peristaltic motion controled by AUERBACH's plexus physiologically. But among the many reports on the anatomy, physiology and histology of AUERBACH's plexus, the author have never seen a single report describing its transplantation.

Accordingly, the author carried out the transplantation experiments of AUERBACH's plexus in the small intestine to the bladder wall and ovary, and the attitude of the transplanted ganglion cells in the plexus were studied histologically.

II MATERIALS AND METHODS

Operations were performed as follows; Laparotomy was carried out under general anesthesia with the injection of isomytal sodium. The sparrow egg-sized serous membrane of the small intestine was peeled in the portion 1 meter oral from the ileum end so that the surface of muscle layer was exposed, and the naked muscle layer was sutured to the base of the bladder, 2-4 weeks after the operation, laparotomy was again performed. As stiff adhesion was found between the bladder and the intestine, it was peeled from the intestine side so as to leave the muscle layers of the intestine on the base of the bladder. The part of the intestine, from which the muscle layers were removed, was resected and a side to side anastomosis was carried out.

Thus the transplantation of AUERBACH's plexus of the intestine together with the muscle piece was completely performed, because it was perfectly isolated from the mesenteric blood vessels and nerves.

The muscle piece of the intestine was removed together with the adhered bladder wall as a mass 1, 3 and 6 months after the transplantation respectively and was used as a specimen.

The ovary specimens were obtained by the same method.

All the materials used in this study were obtained from adult dogs. The author used only fresh specimens which were resected operatively. After fixation for 3-4 weeks in 10% neutral formal solution, the specimens were frozen, sliced in thickness of 30-40 μ , fixed again 10% neutral formal solution for more than 2-4 months, and then stained. The author recognized AUERBACH's plexus which was stained with Suzuki's modification of BIELSCHOWSKY's silver impregnating method.

III MICROSCOPIC OBSERVATION OF THE TRANSPLANTED AUERBACH'S PLEXUS OF THE SMALL INTESTINE ON THE URINARY BLADDER AND OVARY

AUERBACH's plexus is a nerve tissue in the connective tissue space between the ring and longitudinal muscles of the intestine wall, and it consists of nerve cells and nerve fibers. The structure of AUERBACH's plexus is divided into 3 portions, i. e. the primary, the secondary, the tertiary plexus. The primary plexus consists of many ganglion cells and nerve fibers, and is localised between the circular and longitudinal muscle layers. The secondary plexus consists of a few ganglion cells and fine nerve fibers. It arises from the primary plexus, enters the muscle layers, and is localised in the connective tissue between muscle fibers. The tertiary plexus ramifies from the secondary plexus, consists of fine nerve networks, and distributes to each muscle fiber.

Nerve cells usually exist in the primary plexus, and they have been differentiated into two types by DOGIEL (1895). Type I nerve cell has many short axons and a few long axons, the formers terminate in the nerve cell capsule without passing through it, while the latter form the neurites, traversing the

capsule. Type I nerve cell mainly has plural axons, sometimes as many as 4-5 axons. Type II nerve cell has both long and short axons, however both of them run out of the capsule. Short axons in type II nerve cell are far less in number than in type I. Most of them ramify and terminate in tapering endings. The greater part of the long axons in type II show a plurality such as in type I nerve cell.

As a control of experiments, the author made an adhesion between the small intestine and the bladder wall, without cutting off the mesenteric blood and nerve supply, and adhered part of the intestine was studied.

In the control specimens, almost normal nerve structures of AUERBACH's plexus as mentioned above were observed (Fig. 1-6).

Next, observing the specimens in which the transplantation was carried out, the following findings were obtained :

(A) The AUERBACH's plexus transplanted on the bladder wall

1) At the end of the 1st month

In AUERBACH's plexus, various degrees of degenerations were found in nerve cells; dysharmony of axons, dislocation of nucleus, and fenestrated cell, etc., were observed. In some specimen the number of nerve fibers in AUERBACH's plexus decreased, in another they proliferated, while in some others decrease and increase in the number of nerve fibers were found in different portions of AUERBACH's plexus (Fig. 7-22).

2) At the end of the 3rd month

Mild degenerated nerve cells were found. A few specimens showed decreased number of nerve fibers, but most of specimens showed marked increase of nerve fibers with a vigorous growth toward the scar (Fig. 23-36).

3) At the end of the 6th month

It was hard to discriminate the transplanted muscle piece in the scar, AUERBACH's plexus remained as a carcase, and nerve cells or fibers were scarcely found there (Fig. 37, 38, 39).

(B) The AUERBACH's plexus transplanted on the ovary

1) At the end of 1st month

Severe degenerated nerve cells were observed, and nerve fibers were generally found to have decreased considerably without a tendency to increase (Fig. 40, 41, 42).

2) At the end of the 3rd month

Most of degenerated nerve cells commonly showed up, light staining, falling of the argent affinity, indistinct figures and invisible axons. Nerve fibers were found to have almost disappeared (Fig. 43-48).

IV DISCUSSION

Considering the results of various experiments performed by many investigators from the anatomical or physiological standpoint, the urinary bladder is mainly innervated by pelvic nerves and by hypogastric nerves. The pelvic nerves

belong to the sacral parasympathetic system, and the hypogastric nerves belong to the sympathetic system, while the formers are more dominant in the bladder than the latters, and the pelvic nerves play a leading innervation in the bladder. The ovary is under the innervation of the ovarian plexus, and especially is innervated by sympathetic nerves which are derived from the spinal segments of Th. 10—L. 2 like the kidney and the testis, while the parasympathetic innervation is very poor.

From these facts and neurohumoralism (DALE), it can be assumed that autonomic nerves of the bladder muscles belong to the parasympathetic system, i. e. the cholinergic nerve system, like AUERBACH's plexus. Both are homotype. The autonomic nerves of the ovary belong to the sympathetic system, i. e. the adrenergic nerve system, therefore, AUERBACH's plexus and the ovary are heterotype.

Next, one may think that intramural nerve cells in the digestive tract wall, i. e. cells of AUERBACH's and MEISSNER's plexus, are the starting point of parasympathetic-postganglionic fibers.

Then in the experiments, at the end of the 1st month after the transplantation of AUERBACH's plexus to the bladder, the author recognized a remarkable cell infiltration, various degenerated nerve cells and fibers. It was thought that these degenerations were affected not only by cutting off the blood supply but also by the inflammation, as was expected. In the preparations of the 3rd month after the transplantation, mild degenerated nerve cells and remarkable proliferation of nerve fibers were found. Though the transplantation seemed to be possible from these results, in the preparations of the 6th month, AUERBACH's plexus remained as a carcase, and the nerve cells and fibers had completely disappeared.

Next, in the 1st month after transplantation to the ovary, nerve cells already showed severe degenerations and nerve fibers showed a one sided remarkable decreasing without a tendency to increase. In the 3rd month, nerve cells showed a high degree of degeneration and nerve fibers almost disappeared.

From these results which the author observed, the transplantation of AUERBACH's plexus may be impossible and once degenerated, nerve cells are irreversible.

In regard to the postganglionic fibers, they began to decrease in the 1st month. In the bladder wall which belongs to the cholinergic nerve system, they lived for a long time. They showed once proliferation, and finally in 6 months completely disappeared. In the ovary which belongs to the adrenergic nerve system, they decreased from the beginning without showing any increase, and in 3 months they disappeared.

The postganglionic nerve fibers, which were isolated from the preganglionic fibers and transplanted to other organs, show a different attitude in those organs. In a homotype organ, from the view point of chemical mediator of the innervating nerves, the nerve fibers in the transplanted muscle can be alive for a long time, i. e. for over 3 months, once showing proliferation during this time period. In a

heterotype organ, the nerve fibers in the transplanted muscle decrease the number from the beginning and completely degenerate in 3 months.

V SUMMARY AND CONCLUSION

The author carried out the transplantation experiments of AUERBACH's plexus in the small intestine to the bladder wall and ovary, and observed the attitude of the transplanted ganglion cells in the plexus by neurohistological study. And then he investigated the change of postganglionic fibers which were isolated from the preganglionic fibers.

Summarizing the results of experiments, the following conclusions are obtained.

1) The transplantation of AUERBACH's plexus to the urinary bladder and ovary may be impossible. If it is possible, it will be in the bladder.

2) The postganglionic fibers of the parasympathetic nerve begin to decrease in about one month after the isolation, and then go through different courses by the type of nerve innervation;

(a) In the homotype organ (the bladder) which belongs to the cholinergic nerve system, they live a relatively long time.

(b) In the heterotype organ (the ovary) which belongs to the adrenergic nerve system, they continue to decrease to earlier death.

I wish to express my deepest gratitude toward Assist. Prof. Dr. Ch. KIMURA for his helpful advice and kind guidance throughout this study.

References

- 1) Aiba, A.: Über Bewegung des Dickdarms (in Japanese). Jap. J. Med. Prog., **22**, 1684. 1933
- 2) Bayliss, W. M. and Starling, E. H.: The movement and the innervation of the small intestine. J. Physiol., **26** (1901) 125-138
- 3) Bozier, E.: Reflex peristalsis of the intestine. Am. J. Physiol., **157** (1949), 338-342
- 4) Choda, T.: Die Studien über die Innervation des Dünndarms. Okayama-I. Z., **51**, 1484. 1939
- 5) Dale, H. H. and W. Feldberg.: Chemical transmission at motor nerve endings in voluntary muscle. J. Physiol., **81**: 39
- 6) Feyrter, F.: Über die Pathologie der vegetativen nervösen Peripherie und ihrer ganglionären Regulationsstätten. Mandrich, Wien. 1952
- 7) Fukuda, K.: Human Physiology (Jintai seiri gaku, in Japanese). 212-220. 8. 1949
- 8) Fukui, T.: Histopathological Studies on the Stomach and Intestine (in Japanese). J. Kyoto Pref. Med. Univ., **58**, 735., 1955
- 9) Hase, T.: The Action of vagus nerve on the intestine (Meiso shinkei no chosayo). J. Kyoto Pref. Med. Univ., **24**: 1191 (1938)
- 10) Herrmann, H.: Pathologische Histologie der peripheren vegetativen Nervensystems. Berliner Med. Verlagsanstalt 1956
- 11) Inouye, R.: Pathohistologische Studien über intramuralen Plexus des Colons bei chronischer Obstipation, nebst einigen Bemerkungen zur chirurgischen Indikationsstellung. Arch. f. jap. Chir., **27**, 1341. 1958
- 12) Jabonero, V.: Der anatomische Aufbau des peripheren neurovegetativen Systems. Acta Neuro-veg.; Suppl. IV. Springer. Wien 1953
- 13) Kimura, Ch.: Surgery of the Autonomic Nervous System (Jiritsu Shinkei no Geka, in Japanese). J. Jap. Surg. Soc., **52**, 450. 1951; Nihon Geka Zensho. **9**; 341. 1956
- 14) Kimura, Ch.: The Problem of Abdominal Pain. Arch. f. jap. Chir., **22**, 59. 1953

- 15) Kimura, Ch. : Physiology of Abdominal Pain (Fukutsu no Seiri, in Japanese). Rinsho no Shimpō. 7. 1953
- 16) Kimura, Ch. : Abdominal Pain (Shinkei-Biori to Rinsho, in Japanese). J. Jap. Surg. Soc., 6. 947. 57. 1956
- 17) Kure, T. and Okinaka, S. : Autonomic Nervous System (in Japanese). 1949
- 18) Langley, J. N. : a) The Autonomic Nervous System. Brain, 26, 1. 1903
b) The Autonomic nervous system. Part, I, Cambridge, W. Heffer and Sons Ltd. 1921
- 19) Müller, L. R. : Lebensnerven und Lebenstrieb. 3. Aufl., 1931
- 20) Okinaka, S. : Morphology of autonomic nerve fiber (Jiritsu shinkei sen-i no keitai). Nihon Rinsho., 8. 11. 1950.
- 21) Sato, H. : A Histological Study of the Afferent Innervation of the ovary of the Dog. Arch. f. jap. Chir., 24 ; 456, 1955
- 22) Seto, H. : a) Advance of Medicine. 5, 223, 1949.
b) Histological Observation of Human Sensibility. Rinsho no Nihon. I; 32. 1955
- 23) Stoehr, Jr. Ph. : a) Lehrbuch der Histologie und der Mikroskopische Anatomie des Menschen. Springer Verlag. 1951
b) Mikroskopische Anatomie des vegetativen Nervensystems. 1951 u. 1957
- 24) Shishido, S. : Abdominal Pain (Naizochikaku no Dendoro o chusin to shite, in Japanese). J. Jap. Surg. Soc., 6. 922. 57. 1956
- 25) Suzuki, K. : Note of Technique to make Tissue-Preparations (Soshiki Hyohon Seisaku Gijyutsu Note, in Japanese). (IV). Noshinkei ryoiki, 5. 184. 1952
- 26) Thomas, J. E. and A. Kuntz. : A study of gastro-intestinal motility in relation to the enteric nervous system. Am. J. Physiol. 76 (1926), 606-626
- 27) Takayasu, T. : Histologische Studien über die Innervation der Darmwand beim Menschen. (1) (2) (3) (in Japanese) J. Tokyo Soc. Med. Sci., 48. 837; 1955. 1934. 49. 259 ; 901. 1935.
- 28) Uchino, T. : Studien über die Degeneration der intramuralen Darmnerven bei experimentellem Ileus. (in Japanese) Nagasaki IgKZ., 19, 1959 ; 2423, 1941
- 29) Yoshida, T. : A Histological Study of Sensory Nerves in the Urinary Organs. Arch. f. jap. Chir., 26. 55. 1957

Suzuki's Method

The specimens, after having been sliced with the freezing method and kept in 10% neutral formal solution, are

- 1) Washed 3 times with distilled water, each time for about 10 minutes,
- 2) Put into 20% silver nitrate solution for about 1 hour, in the darkness,
- 3) Washed with distilled water for a few seconds,
- 4) Put into ammonical silver solution until the specimens are colored light yellow,
- 5) Placed in 10% sodium-potassium tartrate solution for a few minutes until the specimens are colored gold yellow,
- 6) Washed with distilled water for a few minutes,
- 7) Placed in 0.05-0.1% gold chloride solution for 1-2 hours,
- 8) Washed with distilled water for a few minutes,
- 9) Placed in 20% sodium thiosulfate solution,
- 10) Washed in distilled water,
- 11) Dehydrated and mounted.

和 文 抄 録

アウエルバッハ氏神経叢の膀胱及び卵巣への移植
並びに移植神経叢の消長に関する組織学的研究

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）

野 村 源 藏

成犬小腸のアウエルバッハ氏神経叢を膀胱及び卵巣へ移植し、移植後1カ月、3カ月、6カ月の標本に於て、移植神経叢の消長並びに節前線維より遮断された副交感神経節後線維に就て検討し、次のような結論を得た。

1) 移植後1カ月、膀胱に於ては著明な細胞浸潤を認め、アウエルバッハ氏神経叢の神経細胞には核偏位、有窓細胞等種々の変性が強く、神経線維は或標本に於て減少し、又他の標本に於ては増殖しているのが認められた。更に神経線維の増殖減少が同一標本に於て異なつた場所に認められることもあつた。これらの変化は血流遮断によるだけではなく、炎症の影響を強く受けていると考えられ、

2) 3カ月の標本に於て、神経細胞の変性は少なく、神経線維は極く少数の標本に於て減少しているが、多くの標本に於ては著しく増殖し瘢痕へ伸びて行くのが認められた。この結果から移植可能と思われたが、

3) 6カ月の標本に於て、移植筋肉片は瘢痕と判別し難く、アウエルバッハ氏神経叢は残骸を残す状態

で、神経細胞及び神経線維は全く消失していた。

4) 次に卵巣への移植は移植後1カ月に於て、既に神経細胞には高度の変性が認められ、神経線維は全く増殖の傾向なく一方的に著しく減少しているのが認められた。

5) 3カ月の標本に於ては、神経細胞の変性が更に高度となり、神経線維は全く消失していた。

6) 以上の結果から、アウエルバッハ氏神経叢の移植は不可能である。若し可能であるとすれば、それは膀胱に於てであろう。

7) 節前線維より遮断され、他の臓器に移植された節後線維は約1カ月内に減少し始め、その後神経支配の型によつて態度を異にする。

- (a) 同型の臓器、即ち向コーリン神経系に属する膀胱に於ては、副交感神経節後線維は比較的長く3カ月以上も生存し、
- (b) 異型の臓器、即ち向アドレナリン神経系に属する卵巣に於ては、副交感神経節後線維は減少の一途を辿り早期に消失する。

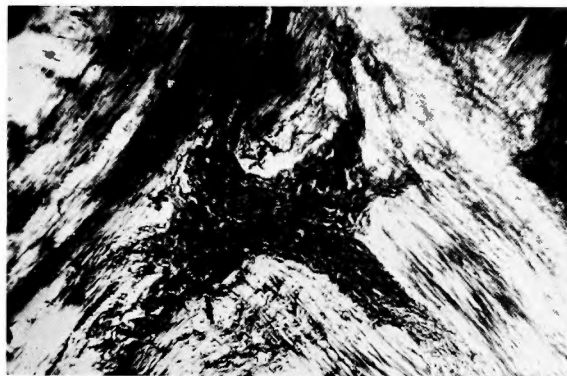


Fig. 1 The control specimen. The primary and secondary plexuses in the AUERBACH's plexus of the small intestine. $\times 100$

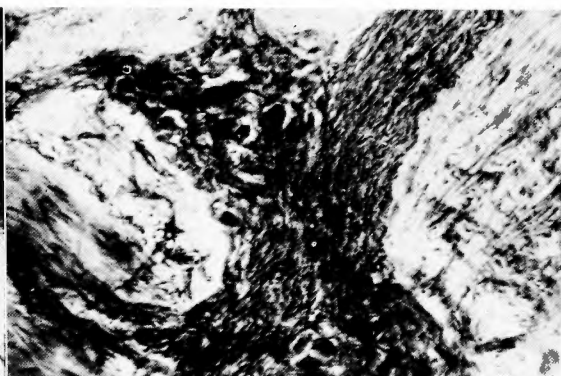


Fig. 2 Enlargement of Fig. 1. The primary plexus. $\times 200$

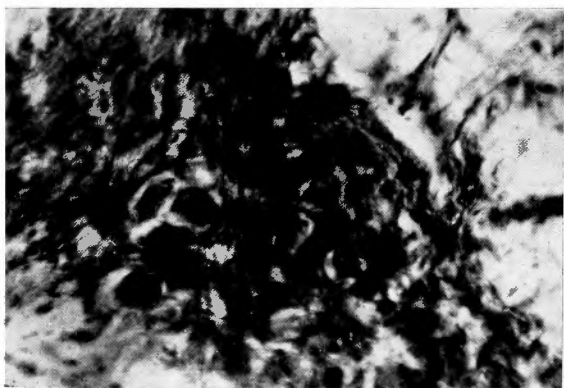


Fig. 3 Enlargement of Fig. 1. Abundant nerve cells and fibers in the primary plexus. $\times 400$

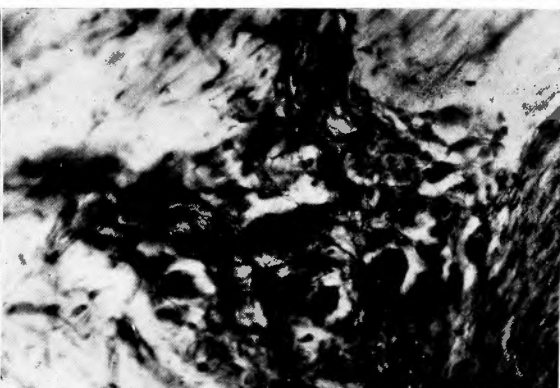


Fig. 4 Enlargement of Fig. 1. The same state as in Fig. 3. $\times 400$



Fig. 5 The control specimen. The primary and secondary plexuses. $\times 100$

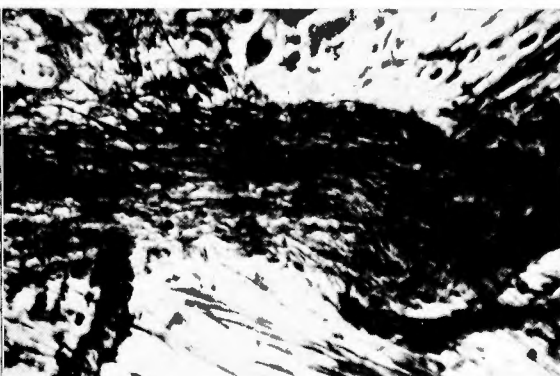


Fig. 6 Enlargement of Fig. 5. Abundant nerve fibers in the AUERBACH's plexus. $\times 400$

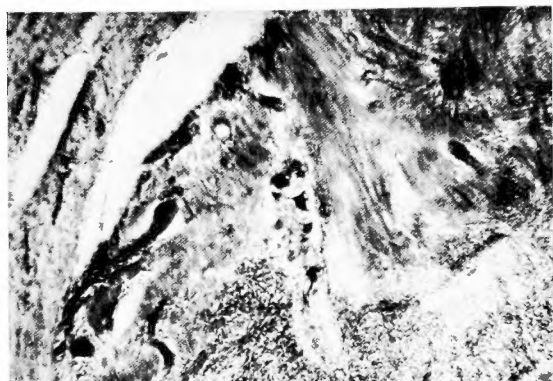


Fig. 7 A notable cell infiltration 1 month after the transplantation of AUERBACH's plexus to the bladder wall. $\times 100$

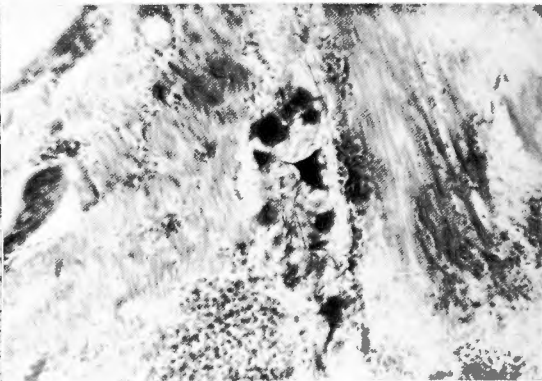


Fig. 8 Enlargement of Fig. 7. $\times 200$



Fig. 9 Enlargement of Fig. 7. Decreased nerve fibers in the AUERBACH's plexus. $\times 400$



Fig. 10 Degenerative changes in the AUERBACH's plexus 1 month after the transplantation to the bladder wall. $\times 200$



Fig. 11 A notable cell infiltration 1 month after the transplantation of AUERBACH's plexus to the bladder wall. $\times 100$

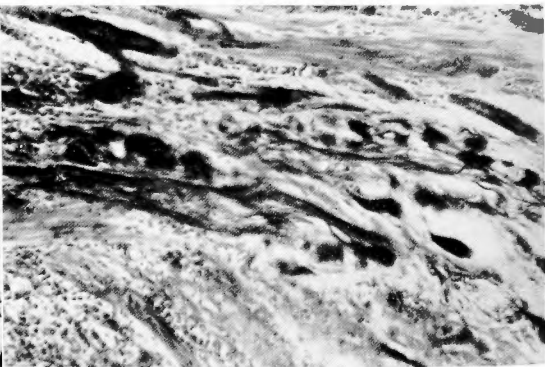


Fig. 12 Enlargement of Fig. 11. Partial proliferation of nerve fibers. $\times 200$

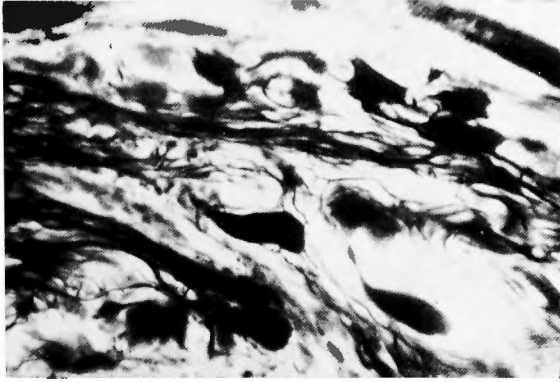


Fig. 13 Enlargement of Fig. 11. Shrinkage and the hyperchromasia of cytoplasm of nerve cells. $\times 400$

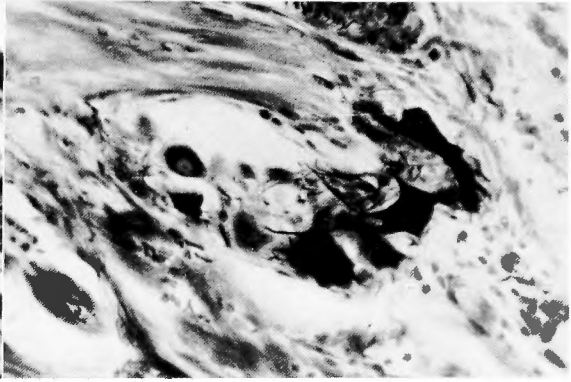


Fig. 14 Dysharmonious axons and decreased nerve fibers of degenerated nerve cells, 1 month after the transplantation to the bladder wall. $\times 400$

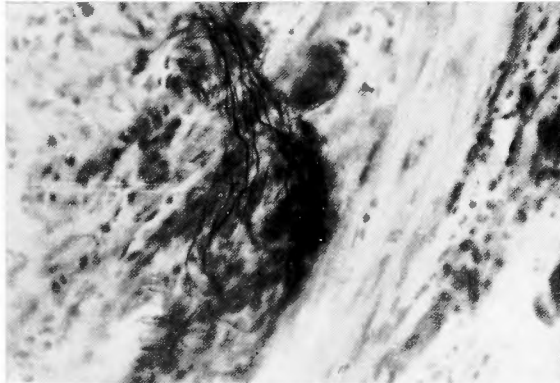


Fig. 15 Decreased nerve fibers 1 month after the transplantation to the bladder wall. $\times 400$

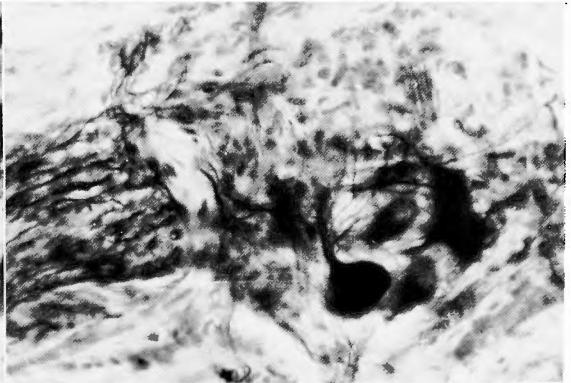


Fig. 16 Decrease of nerve cells and nerve fibers, 1 month after the transplantation to the bladder wall. $\times 400$

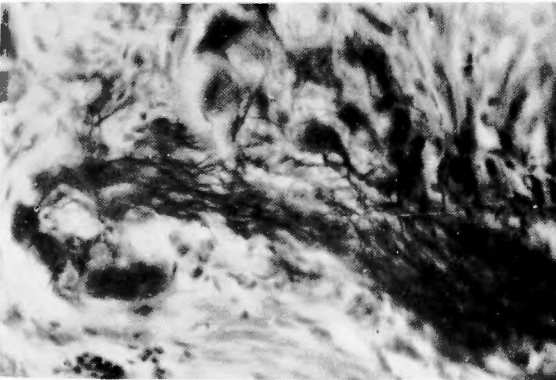


Fig. 17 Slight increase of nerve fibers 1 month after the transplantation to the bladder wall. $\times 400$

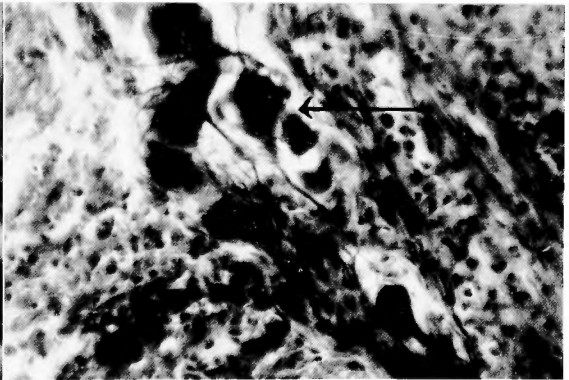


Fig. 18 A fenestrated cell in AUERBACH'S plexus 1 month after the transplantation to the bladder wall. $\times 400$



Fig. 19 Dislocation of nucleus in type I nerve cell (DOGIEL) 1 month after the transplantation to the bladder wall. $\times 900$



Fig. 20 Decrease of the satellite cells of ganglionic cells (DOGIEL type I neuron) and dislocation of nucleus 1 month after the transplantation to the bladder wall. $\times 900$

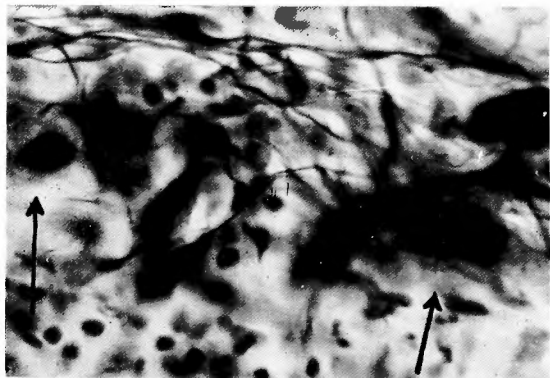


Fig. 21 Normal type II nerve cells (DOGIEL) and shrinkage of cytoplasm in type I nerve cells, 1 month after the transplantation to the bladder wall. $\times 900$



Fig. 22 A type II nerve cell (DOGIEL) remained without a marked change, 1 month after the transplantation to the bladder wall. $\times 900$



Fig. 23 Mild cell infiltration 3 months after the transplantation to the bladder wall. $\times 100$

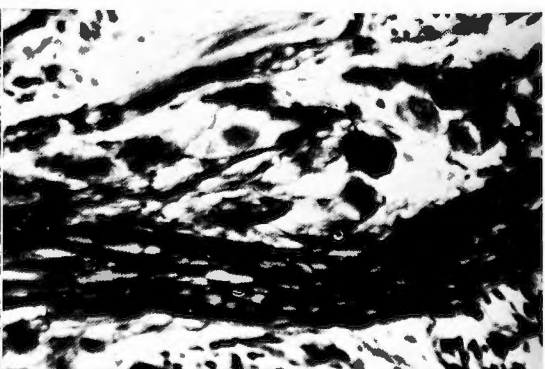


Fig. 24 Enlargement of Fig. 23. Proliferation of nerve fibers. $\times 400$



Fig. 25 Enlargement of Fig. 23.
Strongly increased nerve fibers. $\times 400$



Fig. 26 Nerve cells remaining intact without marked changes, and slight decrease of nerve fibers 3 months after the transplantation to the bladder wall. $\times 400$



Fig. 27 Slight decrease of nerve fibers with mild cell infiltration 3 months after the transplantation to the bladder wall. $\times 100$

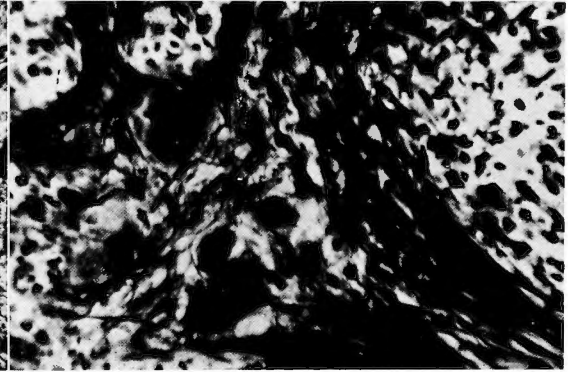


Fig. 28 Enlargement of Fig. 27.
Almost normal AUERBACH's plexus (The primary plexus). $\times 400$

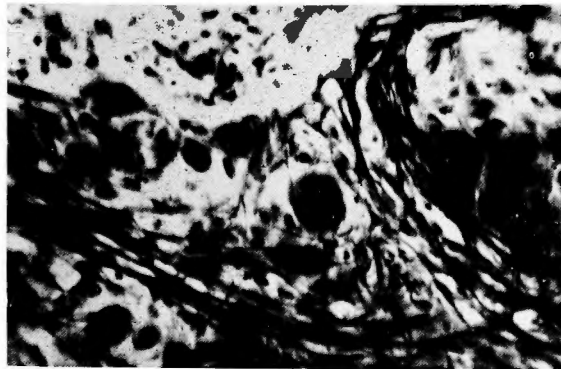


Fig. 29 Enlargement of Fig. 27.
Nerve fibers increase in one portion and decrease in another. $\times 400$

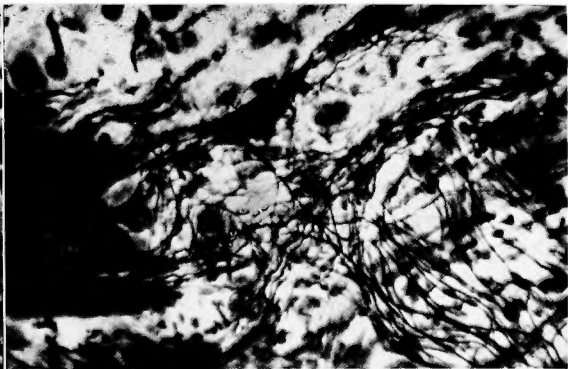


Fig. 30 Enlargement of Fig. 27.
Partial disappearance of nerve cells remaining spaces in the AUERBACH's plexus. $\times 400$

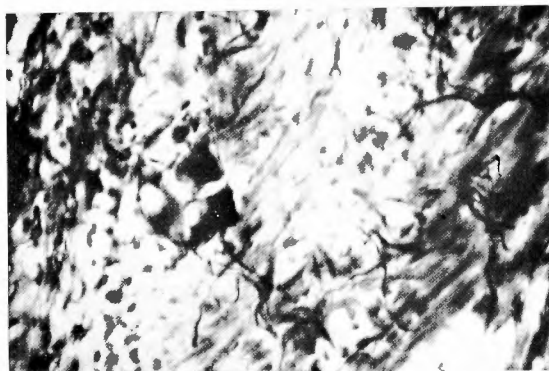


Fig. 31 A notable decrease of nerve cells and fibers 3 months after the transplantation to the bladder wall. $\times 400$

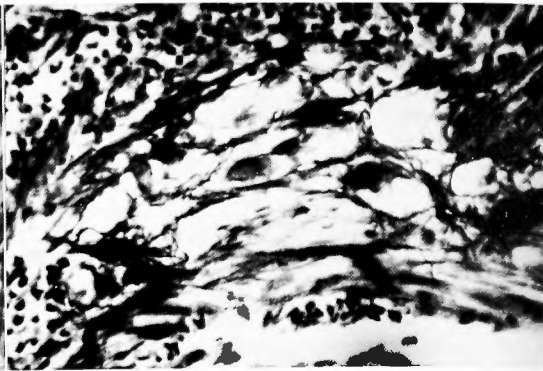


Fig. 32 Shrinkage and transparency of cytoplasm in the the primary plexus 3 months after the transplantation to the bladder wall. $\times 400$

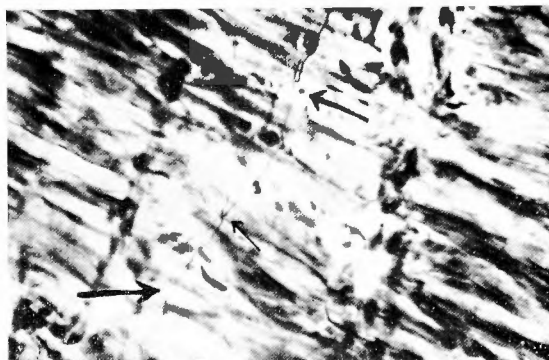


Fig. 33 A fine nerve fiber running toward the muscle fiber (the tertiary plexus) 3 months after the transplantation to the bladder wall. $\times 400$

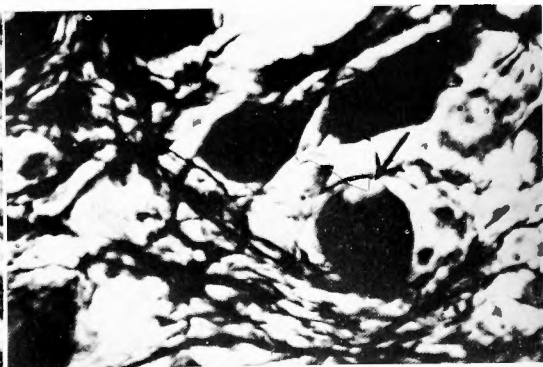


Fig. 34 Nerve cells are stained heavily and one of them has a vacuole, 3 months after the transplantation to the bladder wall. $\times 900$



Fig. 35 Shrinkage of cytoplasm and decrease of nerve fibers 3 months after the transplantation to the bladder wall. $\times 900$

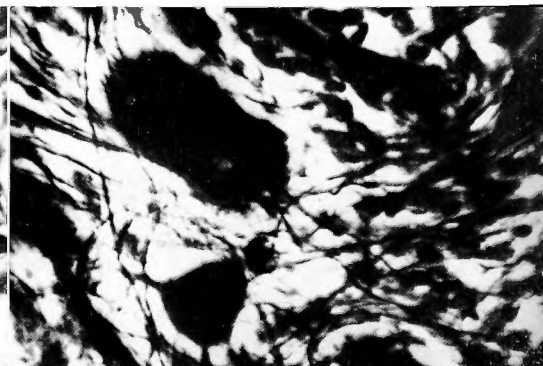


Fig. 36 Nerve cells are stained heavily and partial increase of nerve fibers is shown, 3 months after the transplantation to the bladder wall. $\times 900$



Fig. 37 A carcass of AUERBACH's plexus in the scar, 6 months after the transplantation to the bladder wall. $\times 30$

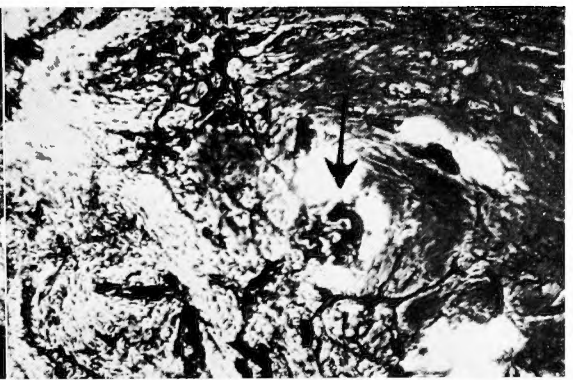


Fig. 38 Enlargement of Fig. 37. $\times 200$

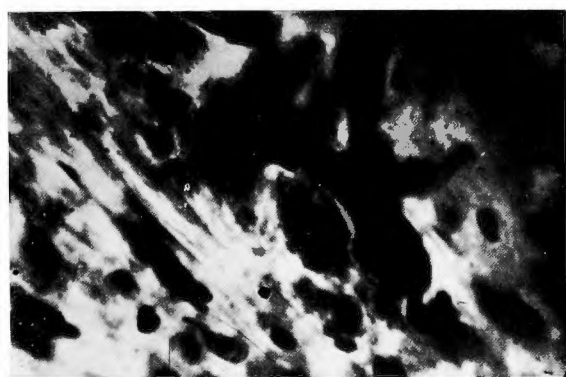


Fig. 39 Enlargement of Fig. 37. Destroyed nerve cells and disappearance of nerve fibers. $\times 900$



Fig. 40 Atrophied AUERBACH's plexus 1 month after the transplantation to the ovary. $\times 100$



Fig. 41 Enlargement of Fig. 40. A considerable decrease of nerve fibers. $\times 400$

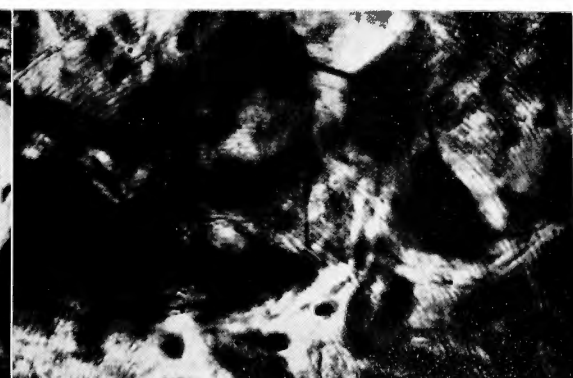


Fig. 42 Enlargement of Fig. 40. The indistinct figure of nerve cells due to degenerations and broken nuclei. $\times 900$

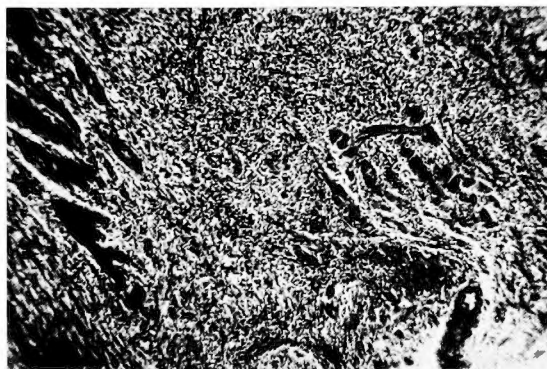


Fig. 43 A notable cell infiltration 3 months after the transplantation to the ovary. $\times 100$

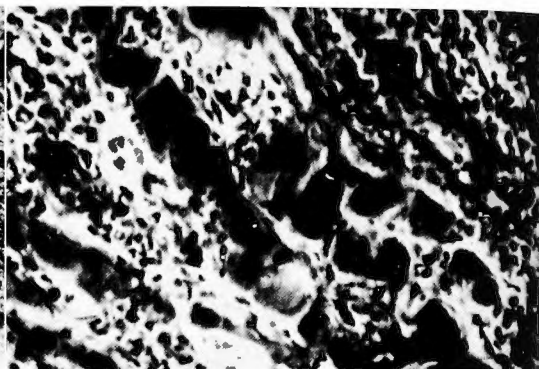


Fig. 44 Enlargement of Fig. 43.
Disappearance of axons and nerve fibers. $\times 400$



Fig. 45 Enlargement of Fig. 43.
Breaking of nucleus in nerve cells. $\times 900$



Fig. 46 Atrophied AUEKBACH's plexus 3 months after the transplantation to the ovary. $\times 100$

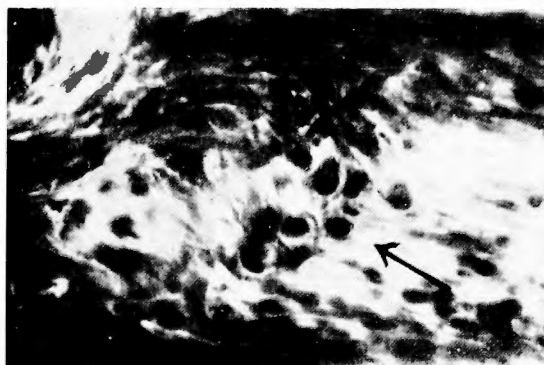


Fig. 47 Enlargement of Fig. 46.
Disappearance of nerve fibers and axons. $\times 400$



Fig. 48 Enlargement of Fig. 46.
A indistinct figure of nerve cells and shrinkage of cytoplasm. $\times 900$