NEUROHISTOLOGICAL STUDY ON NORMAL AND PATHOLOGICAL THYROID GLANDS

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NEUROHISTOLOGICAL STUDY ON NORMAL AND PATHOLOGICAL THYROID GLANDS

by

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I. INTRODUCTION

Though it had been advocated that endocrine glands have no afferent nerve fibers, many colleagues in our laboratory, and others have reported endocrine glands have nerve fibers, which control secretional function. For example, SETO (hypophyseal gland), SETO and UTSUSHI (pancreas), A. SATO (supraadrenal gland), YAMASHITA and OTSUI (testicle), SAKAGUCHI and H. SATO (ovary), and others have reported, from the histological point of view, that nerves exist in the above mentioned organs. It is clinically accepted that pain arises frequently as a symptom of disease of the thyroid gland, for example, HASHIMOTO's disease, RIEDEL's struma and thyroiditis, etc.

SUNDER-PLASSMANN made detailed histological reports about nerves of normal and pathological thyroid glands. According to his schema, nerve fibers clearly penetrate into parenchymatous calls of the thyroid gland, forming “Terminalreticulum” which finally surrounds each acina. The author, however, is doubtful whether all of them are nerve elements.

Many reports on the functional correlation between autonomic nerves and the thyroid gland have been physiologically proved by L.R. MÜLLER, NAKAOKA, TANAI, OKINAKA, SHIZUME and others. But there is no histological report
describing the nature and the course of the nerves which control the thyroid gland.

The author has attempted to investigate the neurohistological picture of normal and pathological thyroid glands and to pursue the innervations of the thyroid gland.

II. MATERIALS AND METHODS

The materials used in the present study were the thyroid glands of human beings and adult dogs. Fresh specimens were taken from the thyroid glands which had been resected by operation or autopsy. They were fixed in 10% neutral formalin solution for 4 weeks. The specimens were frozen, sliced in thickness of 30-40 μ, fixed again in 10% neutral formalin solution for 2-6 months more, and then stained.

The myelin sheath was stained by EHRLICH’s method, while the axis-cylinder was impregnated by SETO’s modification of BIELSCHOWSKY’s method.

In order to determine the sites of roots of the sensory nerves in thyroid glands, degeneration experiments were done as described by KIMURA and OTSU in their paper: “Systematic Observation of the Visceral Sensory Nerves”. Spinal nerves were cut off at the distal side of dorsal root ganglia, the vagus nerve at a point distal to the nodose ganglion in the neck and the nerve fibers in the thyroid glands were stained by EHRLICH’s method to investigate secondary degeneration. According to the experimental results obtained by colleagues in our laboratory, peripheral nerves demonstrated secondary degeneration 5-6 days after section of the roots of the spinal nerve. Therefore, the thyroid glands of dogs were extirpated 5-6 days after rhizotomy. In the vagotomy cases the author took specimens 7-8 days after neurotomy as described in previous reports.

Operations were carried out under general anesthesia with injection of amobarbital sodium (Isomytal) and thiopental sodium (Ravonal). The animals were sacrificed by bleeding of the femoral artery under general anesthesia and specimens were taken immediately.

Section of nerves was done mainly on one side, but neurohistological study was done on both lobes of the thyroid gland.

Operations were performed as follows:

Croup I.
1) Section of the ventral and dorsal roots on the left side (C₄).
2) Section of the ventral and dorsal roots on the left side (C₅-C₆).
3) Section of the ventral and dorsal roots on the left side (C₇-C₈).
4) Section of the ventral roots on the right side (T₁-T₅).
5) Section of the dorsal roots on the right side (T₁-T₂).
6) Section of the dorsal roots on the right side (T₃-T₄).
7) Section of the dorsal roots on the right side (T₅-T₇).
8) Section of the ventral and dorsal roots on the right side (T₅-T₇).

Croup II.
9) Cervical vagotomy on the right side at a point distal to the ganglion
10) Cervical vagotomy on the left side at a point distal to the ganglion nodosum.

As pathological thyroid glands, thyroid cancer (Struma maligna) and exophthalmic goiter (Struma Basedowiana) were studied.

III. EXPERIMENTAL RESULTS

1) Nerves in the normal thyroid gland:

SETO's modification of Bielschowsky's silver impregnation done on the specimens gave the following findings;

Among the nerve fiber bundles (Figs. 1, 2) which penetrate the thyroid gland with blood vessels, vegetative nerves having delicate and equally calibrated fibers go along capillaries within the thyroid gland, passing through interstitial tissues of follicles, finally reaching the subepithelial tissue immediately adjacent to the epithelial cells of follicles, where they form nervous syncytia. They never enter, however, into the epithelial cells of follicles (Figs. 3, 4, 5).

Sensory nerve fibers mentioned by Prof. SETO, run with blood vessels, pass through the interstitial tissues of follicles, reach positions immediately adjacent to the follicle cells and show sensory nerve endings which seem to end freely or in arborized form (Figs. 6, 7, 8, 9). In the same specimen, glomerular sensory nerve endings consisting of two nerve fibers are also observed in the interstitial tissues of follicles (Figs. 10, 11).

Nerve cells have never been observed within the thyroid gland. Observing myelinated nerves by staining with EHRlich's method, numerous myelinated nerves which penetrated the thyroid gland with blood vessels were found. They formed nerve bundles together with non-myelinated nerve fibers and they passed through the interstitial connective tissues around follicles and ended immediately adjacent to follicle cells (Figs. 12, 13). The course of these myelinated nerve fibers are almost the same as those of sensory nerve fibers mentioned above.

Supplement: Nerves in parathyroid glands of adult dogs.

By staining with SETO's modification of Bielschowsky silver impregnating method, in parathyroid glands of dogs nerve fibers were found which showed simple-shaped endings and vegetative nervous syncytia running beside the capillaries (Figs. 14, 15). But in specimens stained by EHRlich's method no myelinated nerve fibers were found within the parathyroid glands of adult dogs.

2) The secondary degeneration of sensory nerves in thyroid glands of adult dogs;

Group I.

1. Section of the ventral and dorsal roots on the left side (C4).

By staining with EHRlich's method, no degenerated nerve fibers were found within the thyroid gland on either lobe.

2. Section of the ventral and dorsal roots on the left side (C5-C6).

Degenerated nerve fibers were found in the connective tissues around follicles on
the left side of the thyroid gland (Fig. 16). No degenerated nerve fibers were found in the right lobe of thyroid gland.

3. Section of the ventral and dorsal roots on the left side (C7-C8). Degenerated myelinated nerve fibers were found in interstitial tissues around follicles of the thyroid gland on the left side (Fig. 17), but none on the right side.

4. Section of the ventral roots on the right side (T1-T5). No degenerated nerve fibers were found within the thyroid gland on either side.

5. Section of the dorsal roots on the right side (T1-T2). Degenerated myelinated nerve fibers were found in connective tissues around follicles in the right lobe (Fig. 18), but none in the left lobe.

6. Section of the dorsal roots on the right side (T3-T4). Degenerated myelinated nerve fibers were found in connective tissues around follicles in the right lobe (Fig. 19). No degenerated nerve fibers were found in the left lobe.

7. Section of the dorsal roots on the right side (T5-T7). No degenerated nerve fibers were found in the thyroid gland on either side.

8. Section of the ventral and dorsal roots on the right side (T5-T7). No degenerated nerve fibers were found in either side of the thyroid gland.

Group II:

9. Cervical vagotomy on the right side at a point distal to the ganglion nodosum.

10. Cervical vagotomy on the left side at a point distal to the ganglion nodosum.

In the specimens of Group II degenerated myelinated nerve fibers were found in the thyroid gland only on the operated side (Figs. 20, 21).

3) Nerves in the pathological thyroid glands.

a) Thyroid cancer (Struma maligna) (Fig. 22)

As YAMADA reported, the author also observed cancer dividing into two parts, i.e., marginal zone and central. In both, nerves were found less than in normal thyroid glands.

1. Changes of nerves in the marginal zone.

Observing by silver impregnating method, nerve fibers with normal appearance were found in the marginal zone, where infiltration of round cells including some cancer cells were observed (Figs. 23, 24). While nerve fibers showing degeneration were also observed among them (Fig. 25) None of them showed the irritable condition nor hyperchromia. Nerves in degenerative change were swollen or broken in places (Figs. 26, 27, 28).

2. Change of nerves in the central region.

In the nerve bundles in the central region of cancer, some were normal (Fig. 29), while others were swollen or broken in places and formed vacuoles (Figs. 30, 31, 32). Some of them showed abnormal winding course (Figs. 33, 34). These adenomatous cancers showed no nervous syncytia in them.
b) Exophthalmic goiter (Struma Basedowiana) (Fig. 35)
In the specimens stained with silver impregnating method, nerve fiber bundles ran along with blood vessels in the interstitial connective tissue around follicles. Some showed an almost normal pattern (Figs. 36, 37), while others showed degenerative change (Fig. 38). Vegetative nervous syncytia showed proliferation and were, therefore, found more easily than in the specimens of normal thyroid (Figs. 39, 40, 41); granular change was observed in them (Fig. 42). Moreover, vegetative nervous syncytia showed degenerative change (Fig. 43).

IV. DISCUSSION

Histological or physiological studies have not clarified the control by autonomic nerves of endocrine glands in detail.

OKINAKA, SHIZUME and others reported interesting studies on the correlation between hormonal secretion of the thyroid gland and autonomic nerves. According to them, stimulation of cervical sympathetic ganglia resulted in increase of thyroid hormone, PBI\textsuperscript{3415}C, in venous blood of the gland.

Autonomic nerves are said to form peripheral networks and never terminate in free endings. STOEHR named it "Terminal-reticulum" and JABONERO "Nerveoes Synctium". SETO found nerves which were histologically different from the autonomic nerves that form networks like vascular capillary networks. KIMURA and his colleagues in our laboratory reported that sensory nerves, detected by SETO, are observable in almost all viscera, i.e., in small intestine, large intestine, bile-duct, gall bladder, liver and kidneys, etc., as well as in esophagus, stomach and duodenum. They clarified the sensory innervation of viscera physiologically as well as histologically.

The author has studied neurohistological pictures of normal and pathological thyroid glands on the basis of these opinions.

Experimental results are as follows;

1. Nerves in the normal thyroid glands.

Studies on nerves in various organs have been reported by many investigators; for instance, SADA and TANAKA in the esophagus, SATO and OTSU in the stomach, UTSUSHI, MAKINO, and OTSU in the duodenum, UTSUSHI and INOUE in the bile-ducts, OTSU and MAKINO in the small intestine and OTSU, MAKINO, WANG, and LEE etc. in the large intestine. All of them reported that nerve fibers were found in the mucous and submucous layer but they never penetrate the parenchymatous cells. KUNTZ and SATO reported histologically that in the ovary nerve fibers do not penetrate the vesicular ovarian follicles of adult dogs. Only E. HAGEN reported that nerve fibers penetrated the vesicular ovarian follicles of adult dogs. OTSUJI did not mention whether nerve fibers enter LEYDIG's cells or not. According to the author's histological observation in normal thyroid glands of human beings and adult dogs, myelinated nerves enter into the thyroid glands together with blood vessels and they are distributed in the connective tissues around follicles immediately adjacent to epithelial cells of
follcicles.

In the specimens stained by Bielschowsky-Seto's method nerve fibers in the thyroid glands are distinguished as sensory and vegetative nerve fibers. Sensory nerve fibers terminate immediately adjacent to parenchymatous cells of follicles, showing glomerular endings or simple free endings. Vegetative nerve fibers form "nervous syncytia" without terminating freely and are found immediately adjacent to parenchymatous cells of follicles. These nerve fibers or nervous syncytia never penetrate the cell bodies.

Sunder-Plassmann stated that "Terminalreticulum" (Stoehr) have protoplasmic contact with the parenchymatous cells of thyroid gland follicles. But according to his experimental results, the present author cannot agree with Sunder-Plassmann's opinion.

Seto reported that sensory nerves in viscera show simple-shaped or arborized terminations. Weddell reported from the histological point of view that sensory nerve terminations which showed specific endings could be made artificially during specimen preparation. Tsai in our laboratory discovered Pacinian corpuscles in the liver and reported that sensory nerves in viscera have not only free endings but also specific endings.

According to the author's experimental results in thyroid glands, most of sensory nerve fibers have simple-shaped endings or arborized terminations, while some of them have specific endings, too. Both sensory nerve endings (simple-shaped or arborized and specific endings) were found closely associated in the same specimen.

No nerve cells were found within the thyroid gland.

Supplement: Nerves in parathyroid glands of adult dogs.

In the specimens of parathyroid glands of adult dogs stained with Bielschowsky-Seto's method, nerve fibers which terminate in simple-shaped endings, and nervous syncytia were found. The former are thought to be afferent nerves from their neurohistological picture. These nerves run with capillaries which enter the parathyroid gland,

2. Innervation of sensory nerves in thyroid glands adult dogs

Braecker, L. R. Muller, Kuntz, Noniedez, Nakaoa and Tanai, etc. have reported physiological as well as histological control of the thyroid gland by the cervical sympathetic nerves, branches from the vagus nerve and from the glosopharyngeal nerve.

Nakaoa and Tanai showed remarkable change of follicles on operated side after extirpation or electrical stimulation of the cervical sympathetic ganglia, with only slight change on the opposite side. Okinaka and Shizume observed that stimulation of the cervical sympathetic ganglia caused an increase of thyroid hormone in venous blood of the thyroid gland. Tanai observed histologically that the electrical stimulation of the recurrent nerve on one side causes a bilateral gland picture as found in hypofunction. That is to say, the thyroid gland is innervated by vagus nerve, inhibitory, and by cervical sympathetic
nerve, excitatory.

In the author's experiments, the section of the spinal cervical nerve (C5–C8) or the vagus nerve on one side caused secondary degeneration of myelinated nerves only in the lobe on the operated side. The results mean that there are sensory nerves accompanying the sympathetic trunk and the vagus nerve to the thyroid gland. They innervate the thyroid gland only homolaterally. NAKAOKA and TANAI, reported that thyroid glands are innervated bilaterally by the cervical nerve and vagus nerve on one side. However, according to the author's experiments the sensory nerves in sympathetic trunk and vagus nerve never innervate the thyroid gland on the opposite side.

WHITE illustrated in his book that some thoracic nerves also take part in the innervation of the innervation of the thyroid gland in addition to the vagus and the cervical nerves.

In the author's histological investigation in dogs, secondary degeneration was observed in myelinated nerves in thyroid gland after the homolateral posterior rhizotomy (T1–T2) and (T3–T4) on the right side. However, after the section of the dorsal roots on the right side (T5–T7) no secondary degeneration of myelinated nerves was found in the thyroid gland. After section of the ventral roots on the one side (T1–T4) no degenerated myelinated nerves were found in either lobe of the thyroid gland.

The above mentioned facts mean that some sensory nerve fibers arise from the thoracic sympathetic nerves. It has been proved there are sensory nerves which innervate thyroid gland of dogs, i.e. the fibers in the vagus nerve, the cervical sympathetic nerve and the thoracic sympathetic nerve, then the afferent nerves of the thyroid of dogs are derived from spinal segments extending from C5 to T4.

3. Nerves in the pathological thyroid glands.

a) Thyroid cancer (Struma maligna)

In malignant goiter (Struma maligna), i.e., adenomatous cancer of the thyroid gland, nerve fibers were found within cancer nests. Some of these nerve fibers were almost normal. Their courses were corkscrew shaped in the caoer nests. YAMADA stated that degeneration of nerve fibers in cancer must be due to the pressure of cancer tissue displacing them. Nerve fibers which were found in the author's specimen of Struma maligna showed abnormal undulated course which could be caused by cancer infiltration. YAMADA stated that the histological picture of nerve fibers in the inflamed area around gastric cancer was always in the stimulated state. However, in the author's specimens, some nerve fibers in the round cell infiltration around cancer tissue of the thyroid gland had almost normal histology and did not show any stimulated state. YASUMOTO observed swollen and broken abnormal nerve fibers in the specimens of hypertrophic prostate gland and reported that these were undergoing degeneration. YAMADA and SEKIYAMA reported that degenerated nerve fibers as well as normal ones were found in specimens of cancer of the stomach (YAMADA), tongue and rectum.
(SEKIYA). According to YAMADA, in the specimens of gastric cancer, vegetative nervous syncytia show degeneration and disappear in an early stage of cancer. In the author’s specimens of malignant goiter, only a few nerve fibers were found. Most of the nerve fibers in cancer of the thyroid gland were normal except for a very few degenerated ones. However, no vegetative nervous syncytia were observed. The author’s observations on degenerated nerve fibers and vegetative nervous syncytia are identical with that of YAMADA.

b) Exophthalmic goiter (Struma Basedowiana)

LOEB and ARON etc. have clarified that anterior hypophyseal T. S. H. serves as an accelerator of the thyroid’s hypertrophy and hyperfunction. TANIAI described that the cervical sympathetic nerve accelerated hormonal secretion of the thyroid gland and the recurrent nerve depressed it. SHIZUME reported on the basis of physiological experiments that stimulation of cervical sympathetic ganglion accelerated hormonal secretion of the thyroid gland. The author supposed that if nerves play an important role in hormonal secretion of the thyroid gland, as TANIAI and SHIZUME reported, the specimens of exophthalmic goiter must demonstrate proliferation or hypertrophy of peripheral autonomic nervous structures. In fact proliferation and hypertrophy of nervous syncytia were found in addition to normal, degenerated and swollen patterns. The histological studies by MARUTA and MIDORIKAWA etc. reported that the exophthalmic goiter shows a picture of destroyed follicles as well as those in hyperfunction.

The neuropathological pictures in the present study seems somewhat co-incidental with them.

V. SUMMARY AND CONCLUSIONS

The author investigated the neurohistological picture of the normal and pathological thyroid glands of human beings and adult dogs. Then by means of degeneration experiments, the author demonstrated the sites of spinal segments which give rise to the sensory nerves of the thyroid gland.

The experimental results are as follows:

1. The myelinated nerves were found in the thyroid glands of human beings and adult dogs immediately adjacent to the follicles. Observation of the degenerated myelinated nerves after the section of the nerve roots in dogs clarified the existence of afferent nerves in the thyroid gland.

2. Sensory nerves were observed in the interstitial tissues of thyroid gland follicles of human beings and adult dogs. Some of them ended in specific endings, but most of them showed simple or arborized free terminations. Nervous syncytia were observed immediately adjacent to the thyroid follicles, but they never entered parenchymatous cell bodies. No nerve cell was found within the thyroid glands.

3. Investigation of secondarily degenerated myelinated nerves of the thyroid glands proved the existence of small numbers of sensory nerves which arise from
The spinal sensory nerves of the thyroid of dogs arise from the spinal segments between C₅ and T₄.

4. In specimens of adenomatous cancer of the thyroid a few normal sensory nerves were observed. Abnormally winding nerve fibers and degenerated fibers were also found in cancer nests while no nervous syncytia were found in cancer of the thyroid.

5. In the specimens of exophthalmic goiter (Struma Basedowiana), neurohistological change was not uniform; proliferative and degenerative changes were observed on one hand, while normal sensory nerves and nervous syncytia were found on the other hand.

(I am much indebted to Assist.-Prof. Dr. CHUJI KIMURA for his kind guidance throughout this work and I wish to express my deep thanks toward Dr. T. SERRA and Dr. Y. YASUMOTO for their help during these experiments.)

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Fig. 1  Nerve fiber bundles penetrate the thyroid with blood vessel. B-S ×200

Fig. 2  Nerve fibers penetrate the thyroid with blood vessel. B-S ×200

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**Fig. 11 A** A glomerular sensory nerve ending in the interstitial tissue of follicles. B-S ×630

**Fig. 11 B** Sketch of Fig. 11-A

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Fig. 17 Degenerated myelinated nerves in the connective tissue. E ×200

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E; EHRlich's Method
B-S; BIELSCHOSKY-SETO's Method
和文抄録

正常及び病態に於ける甲状腺の神経組織学的研究

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人及び犬に於て正常並びに病態にある甲状腺の神経像を組織学的に検査し、更に犬を用いて甲状腺を支配する神経を系統的に組織学的に追求して次のような結果を得た。

① 人及び犬の甲状腺には有髄神経が存在し、甲状腺組織の直下にまで達している。しかしあ犬の神経幹切断後にあらわれる有髄神経の二次的変性を検出することによって、甲状腺には求心性神経が存在することが証明された。

② 人及び犬の甲状腺の知覚神経は、細胞間関節内に存在し、単純扁平または樹枝状に終わるもの他に、特殊な条状結末形態をとるものもある。但し有髄性特殊小体はみられない。

神経性 Syncytium は甲状腺組織直下に存在するが、細胞上皮内には見られず。また甲状腺のいずこにも神経細胞は見えなかった。

③ 神経幹を切断し、臓器内の有髄神経の二次的変性を追求することによって、組織学的に犬の甲状腺を支配する知覚神経は、迷走神経幹、頸部交感神経幹を通っているもの他に、胸部交感神経幹を通るものもあることが証明された。犬の甲状腺に於ける脊髄性知覚神経は C3 から T4 の間の脊髄断面から支配されている。

④ 悪性甲状腺腫の腫瘍の標本では、一方に正常な知覚神経線維が見えられた他、またこの標本の癌巣内に細行した神経線維が見えられ、変性に陥いたももの見いだされた。神経の腫状構造はいずこにも見えなかった。

⑤ パセドウ氏病性甲状腺腫では正常な知覚神経及び神経の腫状構造が見られる一方、増殖像を示すもの及び変性像を示すものが存在していて、一定の神経線を示さなかったが、この所見は、本疾患の甲状腺には変性した甲状腺組織や機能の亢進した細胞が混在しているためかかもしれない。