Virchows Arch. Abt. A Path. Anat. 361, 195—209 (1973)

Ultrastructural and Biochemical Study of Benign Ganglioneuroma

Masao Yokoyama, Kiyoki Okada, Akihiko Tokue, and Hisao Takayasu

Department of Urology, Faculty of Medicine,
The University of Tokyo (Director: Prof. H. Takayasu). Tokyo, Japan

Received July 16, 1973

Summary. Ultrastructural and biochemical studies were conducted on three ganglioneuromas to elucidate the morphological features in relation to catecholamine synthesis, storage and secretion.

Ganglioneuroma tissues show striking ultrastructural similarity to sympathetic ganglion cells and neurons, being composed of mature ganglion cells, unmyelinated nerve bundles, infrequent myelinated nerve bundles and abundant interstitial elements. Distinct Schwann cells with basement membrane envelope both ganglion cells and nerve processes. Various degrees of morphological differentiations among these tumors are observed e.g. neurofilaments, neurotubules, abundant small clear vesicles (500 Å in size) and large cored vesicles (1000 Å in size). Small cored vesicles (500 Å in size) are scarcely observed.

In two of three tumors studied, catecholamines were detected by chemical assay. Urinary catecholamines were variable. Ultrastructurally most of the ganglioneuromas and ganglioneuroblastomas possess distinct Schwann cells, although a wide spectrum of morphologic variations has been reported.

Since present study revealed the presence of large cored vesicles and existence of catecholamines in ganglioneuroma, it is suggested that the catecholamines are stored in large cored vesicles in the tumor, although this study and current knowledge on sympathetic tissues could not provide unequivocal evidences.

Introduction

The ultrastructure of the ganglioneuroma has been rarely studied because of low incidence. To our knowledge a total of six cases has been reported in four papers (Takahama, 1963; Robertson *et al.*, 1964; Rosenthal *et al.*, 1969; Greenberg *et al.*, 1969). The last two dealt with identical material. The submicroscopic findings described in these reports vary considerably. For example, some authors report distinct Schwann cells in the ganglioneuroma (Takahama, 1963) as well as ganglioneuroblastoma (Gonzalez-Angulo *et al.*, 1965; Staley *et al.*, 1967; Yokoyama *et al.*, 1971), while others (Robertson *et al.*, 1964; Rosenthal *et al.*, 1969; Greenberg *et al.*, 1969) report the absence of Schwann cells in the ganglioneuroma.

Neurogenic tumors are generally associated with catecholamine disorders as is well known in pheochromocytomas. Biochemical studies have revealed that ganglioneuromas may be associated with abnormal catecholamine metabolism (Greenberg and Gardner, 1960; Rosenstein and Engelman, 1963; Hinterberger and Bartholomew, 1969).

Correlated electron microscopic and biochemical studies have demonstrated the relationship between ultrastructure and catecholamine metabolism in pheochromocytoma (Yokoyama and Takayasu, 1969; Tannenbaum, 1970). A similar approach to the study of neuroblastomas (Greenberg *et al.*, 1969; Yokoyama *et al.*,

1971) and ganglioneuroblastomas (Staley *et al.*, 1967; Misugi *et al.*, 1968; Yokoyama *et al.*, 1971) has been also reported. Only one ganglioneuroma, however, was studied in this way (Rosenthal *et al.*, 1969).

Materials and Methods

Surgically excised fresh tissues of three ganglioneuromas were used for this study. For electron microscopy, specimens of the first case were fixed in cold 1% osmium tetroxide solution buffered with sodium veronal and acetic acid at pH 7.4 (Palade, 1952) and specimens from other two cases were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3 (Sabatini *et al.*, 1963) for 3 hours and postfixed in buffered 1% osmium tetroxide solution for 2–3 hours. After dehydration in ethanol and propylene oxide, the tissues were embedded in Epon 812 (Luft, 1961). Thin sections were cut with glass knives on LKB ultorotome, and stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), then observed with a Hitachi HU-11B electron microscope.

Table 1. Clinical data and results of catecholamine assays on urine and tumor in patient with ganglioneuroma

Case		I (A.H.)	II (K.J.)	III (K.T.)
Age and sex		25 yrs M	5 yrs 7 m. F	8 yrs 1 m. F
Location of tumor		Lt adrenal	Rt post. mediast.	Lt. post. mediast.
Weight of tumor		78 g	28 g	35 g
Chromaffine reaction		Negative	Negative	Negative
Tumor CA	A	nd	trace	0.1
(μg/g)	NA	nd	1.4	2.3
	MA	nd	nd	trace
	DA	nd	nd	0.2
Urinary CA	A	0.9	1.0	6.5
(μg/day)	NA	3.5	3.2	25.8
	MA	—	120.0	54.0
	DA	—	trace	nd

M = indicates male; F = female; post. mediast. = posterior mediastinum; A = adrenalin; NA = noradrenalin; MA = total metanephrine; DA = dopamine; nd = not detectable; — = assay not performed.

Catecholamines were assayed fluorimetrically on tumor tissues and on patients' urine according to the metod by Lund (1950) for adrenalin and noradrenalin, Carlsson and Waldeck (1958) for dopamine and Häggendal (1962) for total metanephrine.

Histological diagnosis was established in all the cases, and no recurrence was observed at least for three years after the surgery. Pertinent clinical data and results of catecholamine assays are shown in Table 1.

Results

The three ganglioneuromas show striking similarities in ultrastructural features. These tumors are composed of typical ganglion cells (Figs. 1 and 2), unmyelinated nerve bundles (Figs. 4–9), infrequent myelinated nerve bundles (Fig. 10) and abundant interstitial elements (Fig. 4). The ganglion cell usually contains a large nucleus with one or two prominent nucleoli (Fig. 1). Chromatin

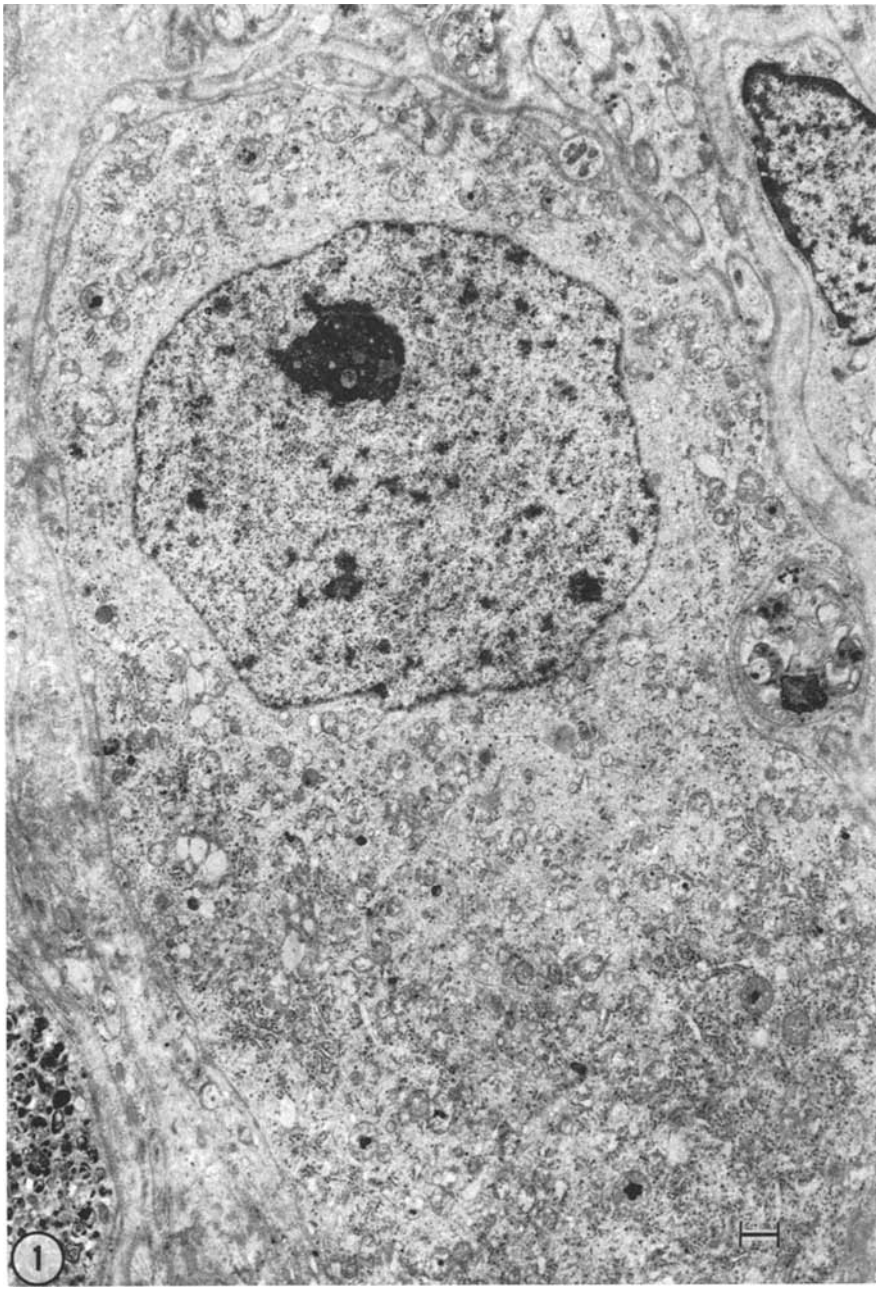

Fig. 1. Ganglion cell surrounded by satellite cell in ganglioneuroma Case III. Note the nucleus with prominent nucleoli and the large cytoplasm-nucleus ratio. Numerous ribosomes, rough endoplasmic reticula, interwoven microtubules and filaments, mitochondria as well as Golgi apparatuses are present. $\times 5000$

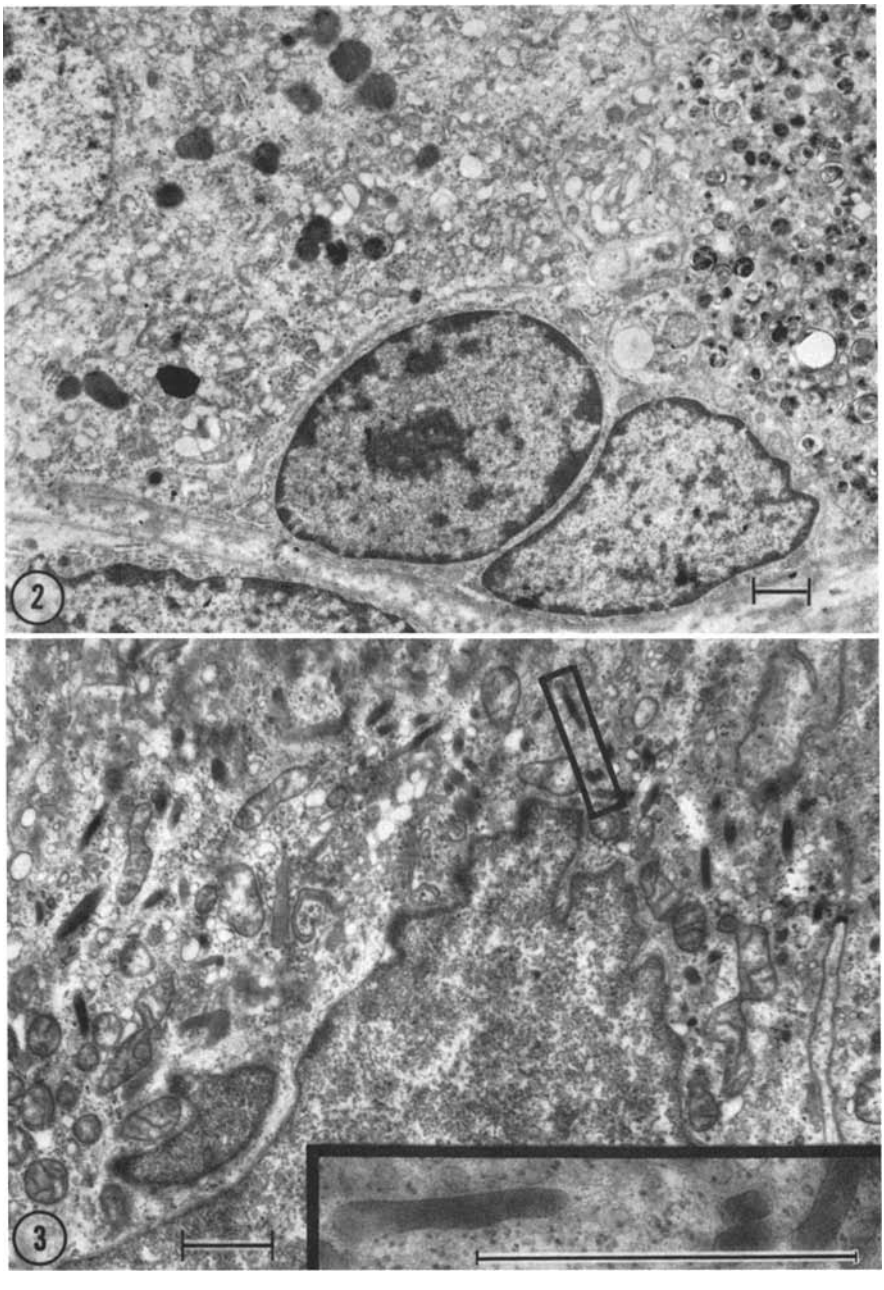

Fig. 2. Two ganglion cells accompanied by a satellite cell in Case III. The ganglion cell on left contains abundant dense bodies, presumably lipofuscin granules, while the cell on right is almost completely occupied by dense bodies of various size with myelin figures inside. Satellite cell is attenuated around the ganglionic cells. × 7500

Fig. 3. Unusual tumor cell found in Case I. Nucleus is bizarre with inconspicuous nucleochromatin distribution. In the cytoplasm elongated structures are randomly distributed. Golgi apparatuses, abundant ribosomes and mitochondria feature the cell. × 12000. Insert: Higher magnification of the elongated dense structures shows limiting membranes and some tubular or fibrillar structures inside. × 50000

is diffusely dispersed throughout the nucleus and shows occasional clumping near the nuclear envelope. Cytoplasm is large in proportion when compared with nucleus and is characterized by the presence of numerous free ribosomes and frequent polysomes, rough endoplasmic reticula, abundant mitochondria, Golgi apparatuses, and plentiful randomly oriented microtubules and microfilaments. Ganglion cells also have cored vesicles and pigment granules, presumably lipofuscin bodies (Fig. 2 left). Some of these cells are filled with numerous membrane bound bodies with myelin figures inside (Fig. 2 right). All the ganglion cells observed in the three cases are surrounded by satellite cells (Figs. 1 and 2), which sometimes envelope not only ganglion cells but nerve processes with attenuated cytoplasmic leaf (Fig. 1). Basement membrane, 300–500 Å in thickness, is present around the satellite cell.

Unmyelinated nerve bundles composed of nerve processes (axons) and Schwann cells are one of the features of the tumor. These bundles appear scattered but interconnect each other among the interstitial tissue. One Schwann cell usually ensheathes several axons (Figs. 4, 5 and 8). Nerve processes are variable in diameter and contain neurotubules and neurofilaments (Figs. 6 and 7), clear and cored vesicles (Figs. 5 and 6), mitochondria (Figs. 5, 6 and 9) as well as inclusion bodies with myelin figures (Fig. 9) which appear similar to those in perikarya of ganglion cells (Fig. 2 right). Axons may be completely (Figs. 4 and 6–9) or partially (Fig. 5 center) surrounded by Schwann cells. Basement membranes around nerve bundles have the same thickness as those around ganglion cells.

Cobbing or widening of axon which contains clear and cored vesicles has been frequently observed. Clear vesicles in the axon of the Case II have a mean diameter of 583 Å $\pm$ 143 Å (mean $\pm$ standard deviation, $n = 82$). Cored vesicles in perikarya or axons measure 1366 $\pm$ 267 Å ($n = 52$) in the same tumor. Typical synapse, however, has not been encountered in any of the three tumors. Small cored vesicles approximately 500 Å in size are rarely observed.

In some part of the unmyelinated nerve bundles, a focal thickening of plasma membrane into a desmosome-like structure has been seen at the axon and the opposing Schwann cell (Fig. 8, arrow). Myelinated nerve bundles are occasionally present in all three tumors. Myelinated axon also contains neurofilaments, neurotubules, mitochondria and inclusion bodies (Fig. 10).

Varying quantities of collagen fibers are interspersed among ganglion cells and nerve bundles (Fig. 4). Fibrocytes and blood vessels are occasionally found. Long-spacing collagen, which has been reported in various tumors of nervous origin (Ramsey, 1965) has not been encountered in the three tumors.

Besides the foregoing findings, this study has discovered some unusual tumor cells in Case I (Fig. 3). These cells have indented nuclei with inconspicuous nucleochromatin and characteristic dense patchy or elongated cytoplasmic organelles. These organelles with limiting membrane and fibrillar or tubular inner structure (Fig. 3, Insert) scatter randomly in the cytoplasm and intermingle with abundant ribosomes, mitochondria and Golgi apparatuses. No satellite cells have been noticed.

Histochemically, the tumor tissue contains no chromaffine material. Biochemically, catecholamines were not detected in tumor tissue from Case I and

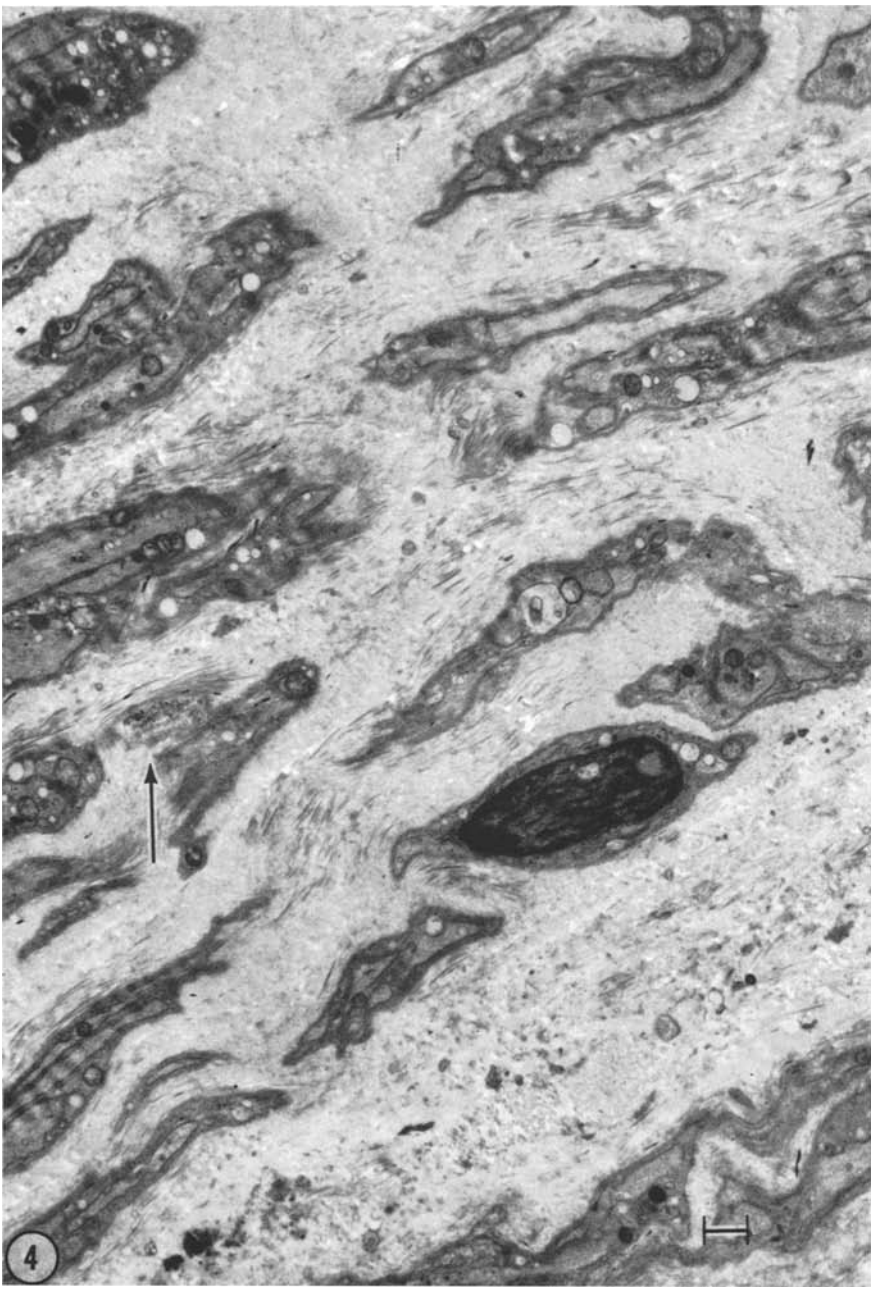

Fig. 4. Low magnification of part of ganglioneuroma Case I. Numerous unmyelinated nerve bundles composed of nerve processes and Schwann cells appear scattered among the interstitial tissue. Axon contains mitochondria, fibrillar structure as well as small vesicles (arrow). Each bundle is surrounded by basement membrane. × 6000

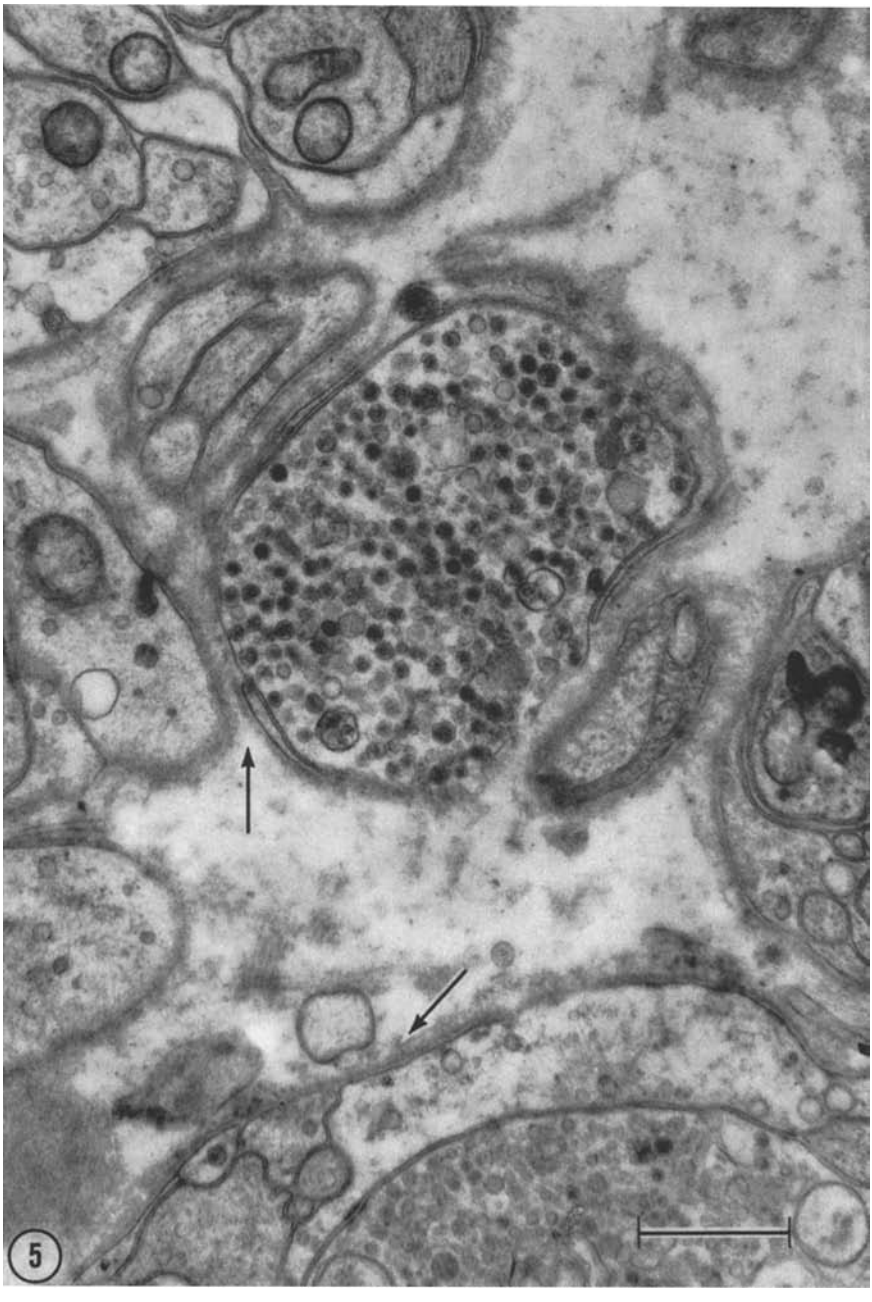

Fig. 5. Unmyelinated nerve process containing numerous cored vesicles in ganglioneuroma Case II. Axons are completely or partially ensheathed by Schwann cells which have distinct basement membrane (arrow). × 20000

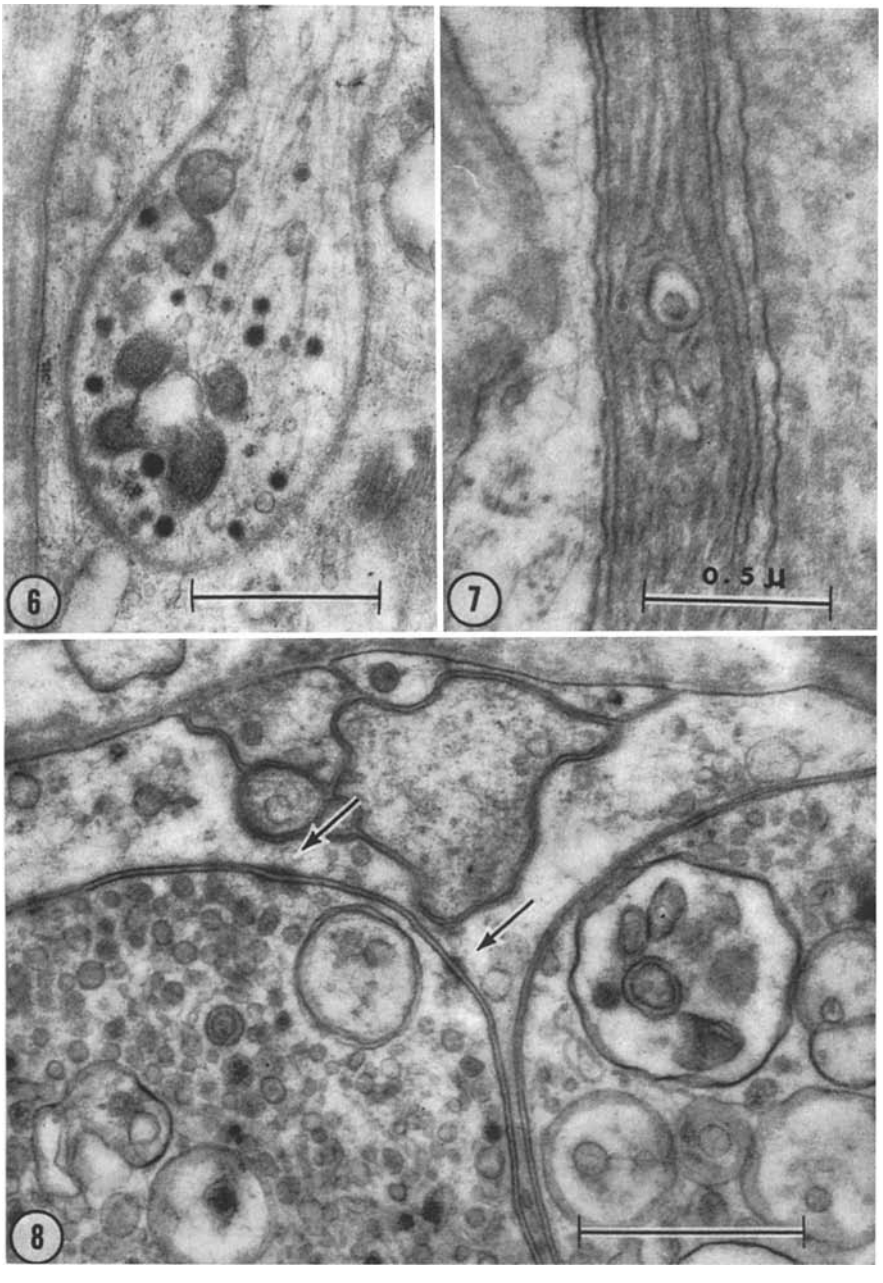

Fig. 6. Part of unmyelinated nerve bundle in ganglioneuroma Case III. Note widening of axon containing cored vesicles, mitochondria and neurotubules. × 25000

Fig. 7. Longitudinal section of an unmyelinated nerve bundle in ganglioneuroma Case II. Note abundant neurotubules. × 50000

Fig. 8. Part of unmyelinated nerve bundle in ganglioneuroma Case II. Two axons contain clear and cored vesicles of various size and membrane bound bodies containing myelin figures. Note the membrane thickenings of Schwann cell and neurite resembling desmosome (arrow). Intercellular space of this particular portion, however, does not show any specialization. × 30000

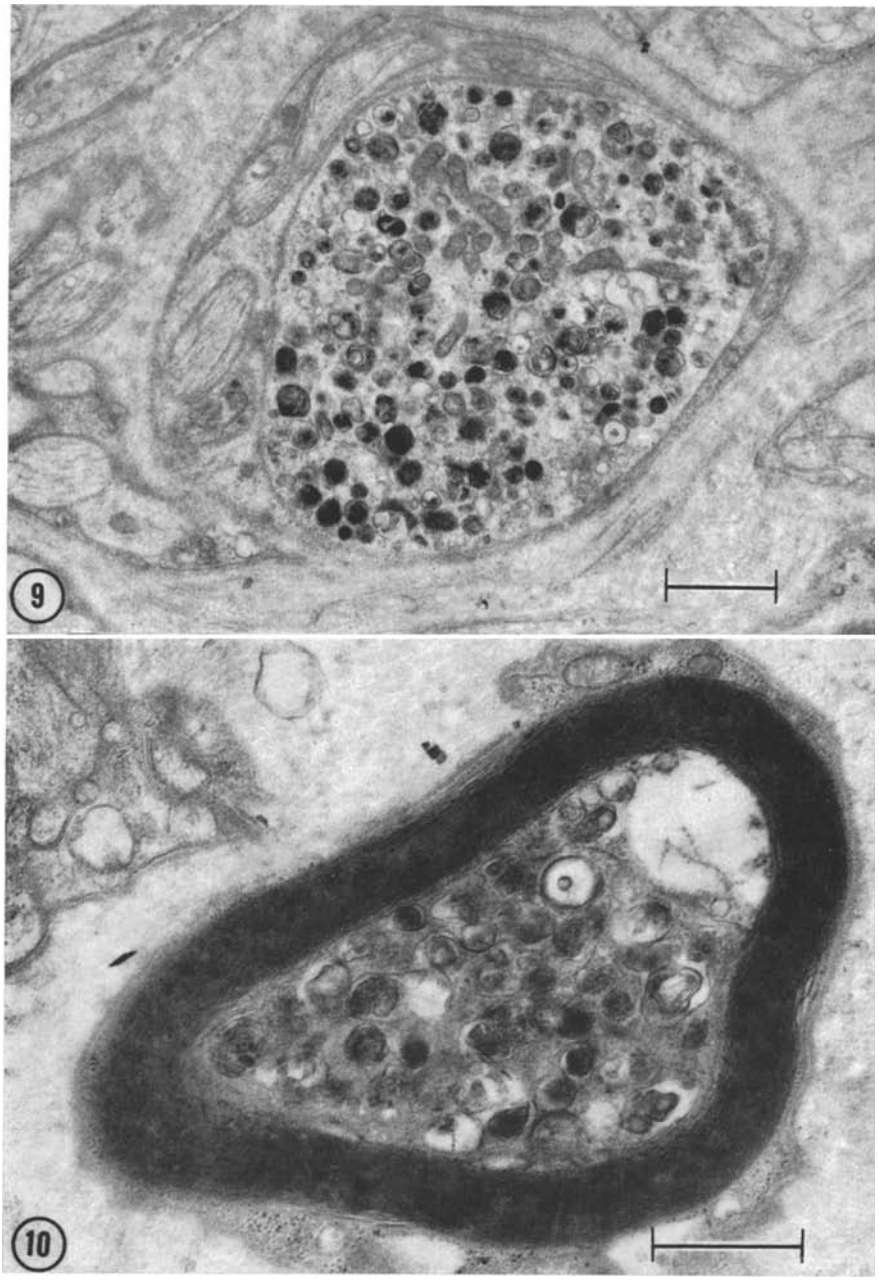

Fig. 9. Unmyelinated nerve bundle in ganglioneuroma Case III. One axon contains normal looking mitochondria and a large number of membrane bound cytoplasmic inclusions with conspicuous myelin figures inside. × 15000

Fig. 10. Myelinated nerve fiber in ganglioneuroma Case I. Axon is filled with membrane bound myelin-like figures (cytoplasmic inclusions) resembling those in unmyelinated axon (Fig. 9) and in ganglionic cell (Fig. 2 right). × 20000

limited amounts of noradrenalin were present in tumor tissues from Case II and III. Tumor from Case III also contained small amount of adrenalin and dopamine. Urinary excretion of the catecholamines was normal in Case I. Increased excretion of noradrenalin was found only in Case III. Urianry metanephrine, however, was elevated in both Case II and III. The data of catecholamine assays on tumor and urine are summarized in Table 1.

Discussion

The present study demonstatred striking similarity between benign ganglioneuromas and mature sympathetic ganglion cells and neurons in man (Cravito, 1962; Pick *et al.*, 1965). Small tumor cells resembling developing ganglion cells (Pick *et al.*, 1964; Tennyson, 1965) reported in a ganglioneuroma (Greenberg *et al.*, 1969) and ganglioneuroblastomas (Gonzalez-Angulo *et al.*, 1965; Staley *et al.*, 1967; Beltran *et al.*, 1969), have not been observed in the three ganglioneuromas.

Submicroscopic findings on ganglioneuromas and ganglioneuroblastomas from various publications including the data reported herein are summarized in Table 2. All the tumors have ganglion cells and numerous nerve processes in various degree of differentiation. Neurofilaments, neurotubules, mitochondria and clear and cored vesicles are present in the nerve processes. When these processes become cob-shaped with membrane specializations and contain abundant vesicles, they give the appearance of synapse.

Seven of nine ganglioneuromas and six of seven ganglioneuroblastomas have been reported to have Schwann cells (Table 2). One ganglioneuroblastoma from metastasis in a lymphnode (Beltran *et al.*, 1969) and an unusual ganglioneuroma of the central nervous system (Robertson *et al.*, 1964) contained no Schwann cells. In both cases nerve processes were not surrounded by basement membrane.

Another ganglioneuroma without Schwann cells has been reported (Rosenthal *et al.*, 1969). Unlike the two tumors described by Beltran *et al.* (1969) and Robertson *et al.* (1964), this tumor has bundles of nerve processes accompanied by basement membrane (Figs. 4 and 5 in Rosenthal *et al.*, 1969). These electron micrographs also show the membrane thickenings identified as desmosomes between axons and dendrites.

The presence of basement membrane around bundles of axons and dendrites without ensheathing Schwann cells seems to be unusual for the following reasons: 1) the basement membrane surrounding nerve bundles has been observed in close association with Schwann cells in most of the ganglioneuromas and ganglioneuroblastomas (Table 2); 2) neuroblastomas which usually contain no Schwann cells, also lack the basement membrane around the tumor cells and cytoplasmic processes (Takahama, 1963; Luse, 1964; Tannenbaum, 1970; Yokoyama *et al.*, 1971); 3) developing ganglion cells have nerve processes, but without encompassing Schwann cells. These nerve processes have also been reported free from basement membrane in rabbit (Tennyson, 1965) and man (Pick *et al.*, 1964). These facts make the intrepretation given by Rosenthal *et al.* of Figs. 4 and 5 in their paper improbable. An alternative interpretation of the electron micrographs could be that nerve bundles are composed of axons and surrounding attenuated Schwann cells accompanied by basement membrane. Desmosome between axon

Table 2. Ultrastructural features of ganglioneuroma and ganglioneuroblastoma

Authors	Takahama	Robertson *et al.*	Rosenthal *et al.*	Present study	Gonzales-Angulo *et al.*	Staley *et al.*	Misugi *et al.*	Beltran *et al.*	Yokoyama *et al.*
Year of Publication	1963	1964	1969		1965	1967	1968	1969	1971
Histological diagnosis	GN	GN	GN	GN	GNB	GNB	GNB	GNB	GNB
No of cases studied	4	1	1	3	2	3	7	1	1
Location	RP Med	CNS	RP	Adrenal Med	RP Med	RP Adrenal Neck	RP Med Neck	Meta	RP
Ultrastructural observation									
Nerve process	+	+	+	+	+	+	+	+	+
Cored vesicles and size	ND	+ 80–150 mu	+ 902 ± 194A°	+ 1366 ± 267 A°	+ 1400 A°	+ 100 mu	+ 1400 A°	+ 70–110 mu	+ 1184 ± 200 Å
Synapse	−	−	+	−	+	−	+	−	+
Desmosome	ND	ND	+	+	−	−	ND	−	−
Myelinated nerve	+	−	−	+	+	ND	ND	−	+
Schwann or satellite cell	+	−	−	+	+	+	+	−	+
Basement membrane	+	−	+	+	+	+	ND	−	+
Immature ganglion cell	−	+	+	−	+	+	ND	+	−

GN = indicates ganglioneuroma; GNB = ganglioneuroblastoma; RP = retroperitoneum; Med = mediastinum; CNB = central nervous system; Meta = metastasis; + = present; − = absent; ND = not described.

and dendrite described by Rosenthal *et al.* appears similar to the desmosome-like structure between axon and Schwann cell in this study (Fig. 8).

Irrespective of this particular case, it is evident that most of the ganglioneuromas and ganglioneuroblastomas have distinct Schwann cells or satellite cells with surrounding basement membrane, although wide variation of morphology is present among the tumors.

A desmosome-like membrane specialization between the axon and the apposing Schwann cell is rare. Desmosomes have been observed in the tissues of non-epithelial origin, e.g. developing thymus of chick (Ackerman and Knouff, 1964), germinal centers of mouse spleen (Swartzendruber, 1965), malignant lymphoma (Swartzendruber and Nelson, 1968), and metastatic melanoma (Mackay *et al.*, 1970).

The presence of myelinated nerve bundles in ganglioneuroma has also been reported by Takahama (1963). The origin of myelinated nerve bundles, however, remains unclear.

Catecholamines were detected by chemical assay in two of our three ganglioneuroma tissues (Case II and III). To our knowledge, chemical determination on catecholamine content in tumor tissue has been conducted only in six cases of the ganglioneuroma (Greenberg and Gardner, 1960; Rosenstein and Engelman, 1963; Gjessing, 1964; Rosenthal *et al.*, 1969; Hinterberger and Bertholomew, 1969). Seven of nine tumors including three in this study have positive catecholamine determinations. Consequently, both ganglioneuromas and sympathetic tissues contain catecholamines and also have similar ultrastructure.

Ultrastructurally sympathetic nerves which store catecholamines contain three kinds of vesicles: arge cored vesicles (1000 Å in size), small cored vesicles and small clear vesicles (both measuring around 500 Å) (Grillo and Palay, 1962). The small cored vesicles are characteristic of the adrenergic nerve endings (Richardson, 1964), and have been proved to contain catecholamines by autoradiography (Wolfe *et al.*, 1962).

Present study shows that the ganglioneuromas have abundant vesicles, mostly small clear vesicles and large cored vesicles. Small cored vesicles are scanty. The observations are in agreement with those reported in ganglioneuroblastoma (Staley *et al.*, 1967), ganglioneuroma (Rosenthal *et al.*, 1969) and neuroblastoma (Yokoyama *et al.*, 1971).

The presence of catecholamine in large cored vesicles has been questioned because cholinergic nerve endings which contain no catecholamine have similar vesicles (Grillo, 1966). In addition, the large cored vesicles fail to incorporate tritiated noradrenalin administered intravenously (Budd and Salpeter, 1969). However, several reports suggest the opposite view. The large cored vesicles are found in ganglionic perikarya (Pick *et al.*, 1965), paraganglion cells (Mascorro and Yates, 1970) and chief cells of carotid body (Chen and Yates, 1970), all of which contain the catecholamines. The effects of pharmacologic agents on large cored vesicles observed in sympathetic nerve endings of pineal gland (Duffy and Marksberg, 1970) and of ciliary ganglion (Huikuri, 1969) also provide suggestive evidences. Recently, two types of vesicles which contain endogenous noradrenalin have been isolated by centrifugation from sympathetic nerve terminals. These two different particles probably correspond to small and large cored vesicles

(Bisby and Fillenz, 1970). Evidently additional critical studies are desirable for determining the specific vesicles which store catecholamines.

Urinary catecholamine assays disclosed dexcessive metanephrine in two patients (Case II and III) and elevated noradrenalin in one (Case III). Despite these results, none of the three patients showed clinical symptoms of excessive catecholamine secretion. Dissociation between clinical symptoms and amounts of urinary catecholamines has been well known in neuroblastoma, ganglioneuroblastoma and ganglioneuroma (Kogut and Kaplan, 1962; Rosenstein and Engelman, 1963).

An attempt was made to seek positive correlation between tumor catecholamine contents, urinary catecholamine excretions and clinical symptoms on the nine ganglioneuromas including three reported herein. From the available data no definite correlation was found. This conclusion could be explained by one or all of the following factors: 1) neurogenic tumor tissues have rapid turnover rate of catecholamines compared to their normal counterpart (Crout and Sjoerdsma, 1964; Greenberg, 1969); 2) tumor tissues have capability to inactivate and degrade catecholamines which are synthesized by themselves (La Brosse and Karon, 1962); 3) biosynthesis of the catecholamines by the tumor are sometimes incomplete (Goldstein, 1966).

The differences in catecholamine storage granules have been demonstrated between the pheochromocytoma and normal adrenal medulla and considered as a reflection of an abnormal catecholamine metabolism in the tumor. The various morphological alterations were also observed and interpreted as manifestations of cellular functional disorders in the tumor (Yokoyama and Takayasu, 1969). In the ganglioneuroma cells reported herein, such morphological implications concerning catecholamine disorders have not been established.

The authors wish to thank Dr. R. Yamada, Department of Internal Medicine, Faculty of Medicine, The University of Tokyo for his help on catecholamine assays; Drs. T. Denda, S. Sawaguchi, and K. Otaguro, Departments of Surgery and Urology, National Children Hospital, Tokyo, for the surgical specimens of Case II and III. Appreciations are also due to Dr. Jeffrey P. Chang, Professor of Biology, Department of Pathology, The University of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Texas for his critical review of this manuscript.

References

Ackerman, G.A., Knouff, R.A.: Lymphocyte formation in the thymus of the embryonic chick. Anat. Rec. **149**, 191–216 (1964)

Beltran, G., Leiderman, E., Stuckey, W.J., Jr., Ferrans, V.J., Mogabgab, W.J.: Metastatic ganglioneuroblastoma: Ultrastructural, histochemical, and virological studies in a case. Cancer (Philad.) **24**, 552–559 (1969)

Bisby, M.A., Fillenz, M.: Isolation of two types of vesicles containing endogenous noradrenaline in sympathetic nerve terminals. J. Physiol. (Lond.) **210**, 49–50 (1970)

Budd, G.C., Salpeter, M.M.: The distribution of labeled norepinephrine within sympathetic nerve terminals studied with electron microscope radioautography. J. Cell Biol. **41**, 21–32 (1969)

Carlsson, A., Waldeck, B.: A fluorimetric method for the determination of dopamine (3-hydroxytyramine). Acta physiol. scand. **44**, 293–298 (1958)

Chen, I-Li. Yates, R.D.: Electron microscopic radioautographic studies of the carotid body following injection of labeled biogenic amine precursors. J. Cell Biol. **42**, 794–803 (1969)

Cravioto, H.: Elektronen mikroskopische Untersuchungen am sympathischen Nervensystem des Menschen. I. Nervenzellen. Z. Zellforsch. **58**, 312–330 (1962)

Crout, J.R., Sjoerdsma, A.: Turnover and metabolism of catecholamines in patient with pheochromocytoma. J. clin. Invest. **43**, 94–102 (1964)

Duffy, P.E., Markesbery, W.R.: Granulated vesicles in sympathetic nerve endings in the pineal gland: Observations on the effects of pharmacologic agents by electron microscopy. Amer. J. Anat. **128**, 97–116 (1970)

Gjessing, L.R.: Studies of functional neural tumors. VI. Biochemical diagnosis. Scand. J. clin. Lab. Invest. **16**, 661–669 (1964)

Goldstein, M.: Enzyme controlling the biosynthesis of catecholamines. In: Neuroblastomas. Biochemical studies (ed. by Bohuon, C.). p. 66–70. Berlin-Heidelberg-New York: Springer 1966

Gonzalez-Angulo, A., Reyes, H.A., Reyna, A.N.: The ultrastructure of ganglioneuroblastoma; Observations on neoplastic ganglion cells. Neurology (Minneap.) **15**, 242–252 (1965)

Greenberg, R., Rosenthal, I., Falk, G.S.: Electron microscopy of human tumors secreting catecholamines: Correlation with biochemical data. J. Neuropath. exp. Neurol. **28**, 475–500 (1969)

Greenberg, R.E., Gardner, L.I.: Catecholamine metabolism in a functional neural tumor. J. clin. Invest. **39**, 1729–1736 (1960)

Grillo, M.A.: Electron microscopy of sympathetic tissues. Pharmacol. Rev. **18**, 387–399 (1966)

Grillo, M.A., Palay, S.L.: Granule containing vesicles in autonomic nervous system. Proc. 5th Intern. Congr. Electron Microscopy at Philadelphia. vol. 2. U-l. New York: Academic Press 1962

Häggendal, J.: Fluorimetric determination of 3-O-methylated derivatives of adrenalin and noradrenalin in tissue and body fluids. Acta physiol. scand. **56**, 258–266 (1962)

Hinterberger, H., Barholomew, R.J.: Catecholamines and their acidic metabolites in urine and tumour tissue in neuroblastoma, ganglioneuroma and pheochromocytoma. Clin. chim. Acta **23**, 169–175 (1969)

Huikuri, K.: Electron microscopic observations on the granular vesicles in the ciliary ganglion of the rat. Experientia (Basel) **25**, 1067–1068 (1969)

Kogut, M.D., Kaplan, S.A.: Systemic manifestation of neurogenic tumours. J. Pediat. **60**, 694–704 (1962)

La Brosse, E.H., Karon, M.: Catechol-O-methyltransferase activity in neuroblastoma. Nature (Lond.) **196**, 1222–1223 (1962)

Luft, J.H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961)

Lund, A.: Simultaneous fluorimetric determinations of adrenalin and noradrenalin in blood. Acta pharmacol. (Kbh.) **6**, 135–146 (1950)

Luse, S.A.: Synaptic structures occurring in a neuroblasoma. Arch. Neurol. (Chic.) **11**, 185–190 (1964)

Mackay, B., Lichtiger, B., Tessmer, C.F.: Observations on the fine structure of metastatic malignant melanoma. Proc. 28th Annual Meeting Electron Microscopy Society America, p. 234–235, 1970

Mascorro, J.A., Yates, R.D.: Microscopic observation on abdominal sympathetic paraganglia. Tex. Rep. Biol. Med. **28**, 59–68 (1970)

Misugi, K., Misugi, N., Newton, W.A.: Fine structural study of neuroblastoma, ganglioneuroblastoma and pheochromocytoma. Arch. Path. **86**, 160–170 (1968)

Palade, G.E.: A study of fixation for electron microscopy. J. exp. Med. **95**, 285–298 (1952)

Pick, J., Gerdin, C., Lemos, C. de: An electron microscopical study of developing sympathetic neurons in man. Z. Zellforsch. **62**, 402–415 (1964)

Pick, J., Lemos, C.de, Gerdin, C.: The fine structure of sympathetic neurons in man. J. comp. Neurol. **112**, 19–68 (1965)

Ramsey, H.J.: Fibrous long-spacing collagen in tumors of the nervous system. J. Neuropath. exp. Neurol. **24**, 40–48 (1965)

Reynolds, E.S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17**, 208–212 (1963)

Richardson, K.C.: The fine structure of the albino rabbit iris with special reference to the identification of adrenergic and cholinergic nerves and nerve endings in its intrinsic muscles. Amer. J. Anat. **114**, 173–205 (1964)

Robertson, D.M., Hendry, W.S., Vogel, F.S.: Central ganglioneuroma: A case study using electron microscopy. J. Neuropath. exp. Neurol. **23**, 692–705 (1964)

Rosenstein, B.J., Engelman, K.: Diarrhea in a child with a catecholamine-secreting ganglioneuroma. Case report and review of the literature. J. Pediat. **63**, 217–226 (1963)

Rosenthal, I.M., Greenberg, R., Kathan, R., Falk, G.S., Wong, R.: Catecholamine metabolism of a ganglioneuroma: Correlation with electron micrographs. Pediat. Res. **3**, 413–424 (1969)

Sabatini, D.D., Bensch, K., Barrnett, R.J.: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. **17**, 19–58 (1963)

Staley, N.A., Polesky, H.F., Bensch, K.G.: Fine structural and biochemical studies on the malignant ganglioneuroma. J. Neuropath. exp. Neurol. **26**, 634–653 (1967)

Swartzendruber, D.C.: Desmosomes in germinal centers of mouse spleen. Exp. Cell Res. **40**, 429–432 (1965)

Swartzendruber, D.C., Nelson, B.: Electron microscopic study of a malignant lymphoma. 1968 Research Report, Medical Division, Oak Ridge Associated Universities. p. 33–37, 1968

Takahama, M.: Electron microscopic study of malignant and benign tumors of the human soft tissue: 1. Tumors of the peripheral nervous tissues. Bull. Tokyo med. dent. Univ. **10**, 281–331 (1963)

Tannenbaum, M.: Ultrastructural pathology of adrenal medullary tumors. In: Pathology annual (ed. by Sommers, S. C.), vol. 5, p. 145–171. New York: Appleton-Century-Crofts 1970

Tennyson, V.M.: Electron microscopic study of the developing neuroblast of the dorsal root ganglion of the rabbit embryo. J. comp. Neurol. **124**, 267–318 (1965)

Watson, M.L.: Staining of tissue sections for electron microscopy with heavy metals. J. biophys. biochem. Cytol. **4**, 475–478 (1958)

Wolfe, D.E., Potter, L.T., Richardson, K.C., Axelrod, J.: Localizing tritiated norepinephrine in sympathetic axons by electron microscopic autoradiography. Science **138**, 440–442 (1962)

Yokoyama, M., Okada, K., Tokue, A., Takayasu, H., Yamada, R.: Ultrastructural and biochemical study of neuroblastoma and ganglioneuroblastoma. Invest. Urol. **9**, 156–164 (1971)

Yokoyama, M., Takayasu, H.: An electron microscopic study of the human adrenal medulla and pheochromocytoma. Urol. int. (Basel) **24**, 79–95 (1969)

Dr. M. Yokoyama
Dr. K. Okada
Dr. A. Tokue
Prof. Dr. H. Takayasu
Department of Urology
Faculty of Medicine
The University of Tokyo
Hongo 7-3-1, Bunkyo-ku
Tokyo, Japan