

HISTOCHEMICAL STUDIES ON CHOLINESTERASE IN PACINIAN CORPUSCLES REPORT II

by

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I. INTRODUCTION

In the previous report, it was stated that cholinesterase (ChE) activity in PACINIAN corpuscles of the cat's mesentery was demonstrated in the central core (Fig. 1), and obstruction of the blood supply or congestion did not cause marked change in ChE activity, however in proportion to the dosage it was reduced by the administration of D.F.P.

In the present report, the author investigated whether ChE in PACINIAN corpuscles was the specific or non-specific type, and noted the change in ChE activity after certain surgical procedures i. e. vagotomy, posterior rhizomy, or section of the sciatic and femoral nerve.

II. MATERIALS AND METHODS

Adult cats were used as the experimental animal. Under general anesthesia with ether operation on various nerves was carried out, and 1-3 weeks after the operation, the cats were sacrificed by bleeding.

PACINIAN corpuscles distributed in the mesentery and in the subcutaneous tissue of hairless pads of cats were used as the subject.

KOELLE's histochemical method (1951) was employed as in the former report to demonstrate the ChE activity. By this technique ChE in the tissue was dyed to yellowish brown.

A) Non-specific ChE in PACINIAN corpuscles.

Butyrylthiocholin (Butchch), the substrate described by KOELLE (1951), was employed.

B) ChE activity in PACINIAN corpuscles after posterior rhizotomy and bilateral vagotomy.

(1) Posterior rhizotomy

The spinal cord was exposed by laminectomy, and the dorsal roots were sectioned bilaterally at the distal portion of the ganglia.

SHEEHAN (1932) has proved that visceral afferent nerves, which reach the PACINIAN corpuscles in the mesentery of the cat have their cell bodies in the spinal ganglia of the dorsal roots of the spinal cord, and most of them are distributed

from the 7th to the 12th thoracic ganglia. By posterior rhizotomy of segments 8 to 11 of the thoracic dorsal roots, most fibers which reach the mesenteric PACINIAN corpuscles may be degenerated.

Likewise in this experiment, to minimize the operative trauma, posterior rhizotomy was performed on the segment from the 8 to the 11 of the thoracic dorsal roots. 1, 2 and 3 weeks after the operation, each group consisting of 3 cats was sacrificed by bleeding, and all visible PACINIAN corpuscles in the mesentery were dissected.

(2) Bilateral vagotomy was performed at the subphrenic portion of the vagal nerve. 1, 2 and 3 weeks after the operation, each group was sacrificed by bleeding, and all visible PACINIAN corpuscles in the mesentery were removed.

C) ChE activity in the degenerated nerve.

Sciatic nerves of cats were sectioned under general anesthesia with ether. 1, 2 and 3 weeks after the operation, the distal portion of the nerve was resected for observation.

All fresh specimens were frozen and cut with the microtome in 30-40 μ , and placed immediately on the cover glasses and kept in the refrigerator for 12-24 hours. The excessive moisture was evaporated, and then the next procedure of KOELLE's method was done.

D) ChE in PACINIAN corpuscles distributed in the subcutaneous tissue of the cat's hairless pads.

In the normal state and 3 weeks after sectioning the sciatic and femoral nerves, the pads were resected for observation. Frozen sections were cut, and the slices were placed on the cover glasses in the cold water, kept in the refrigerator for 12-24 hours and were stained.

III. RESULTS

A) By KOELLE's method, non-specific ChE stained as densely as total ChE in PACINIAN corpuscles, therefore it is assumed that a large part of ChE in PACINIAN corpuscles belongs to the non-specific type (Fig. 2, 9).

B) 1-3 weeks after the posterior rhizotomy (Th 8-11) and bilateral vagotomy, remarkable change in ChE activity in innervated PACINIAN corpuscles was not demonstrated (Fig. 3-Fig. 10).

C) 1) 1 week after sectioning the sciatic nerve, no remarkable change in ChE activity in the nerve was obtained, while the irregularity of the nerve fiber showed marked WALLERIAN degeneration (Fig. 13 a, b)

2) 2-3 weeks after sectioning the sciatic nerve, ChE activity in the nerve fiber was markedly reduced, and the yellowish brown color became very faint. On the contrary, proliferated SCHWANN's cells were stained with hematoxylin over the entire specimen (Fig. 13 c).

D) In PACINIAN corpuscles distributed in the subcutaneous tissue of the cat's hairless pad, ChE activity was demonstrated, as well as in the mesenteric PACINIAN corpuscles. 3 weeks after sectioning both the sciatic and femoral nerve, no marked

change of ChE activity in the innervated subcutaneous PACINIAN corpuscles was detected (Fig. 11, 12).

IV. DISCUSSION

1) It is recognized that a large part of ChE in the nervous system belongs to specific ChE, but non-specific ChE is partly distributed in the myelin sheath, as well as in the glia cells.

As previously mentioned, ChE activity in PACINIAN corpuscles is the nonspecific type.

The physiological role of non-specific ChE in the nervous system is not yet explained, but from the fact that a large part of the ChE in PACINIAN corpuscles belongs to the non-specific type, and that some authors reported on the non-specific ChE in the subcutaneous MEISSNER corpuscles (HILLMAN 1955, BECKETT 1956, UONO 1957), non-specific ChE may be assumed to play some role in the mechanism of sensory transmission.

UONO (1957) demonstrated ChE activity in the taste buds, and SETO (1957) et al. supposed that in the sensory receptor the terminal axons are surrounded by the special cells which are SCHWANN's cell origin, and a chemical substance secreted from these cells by physical stimulus is able to evoke the impulses at the terminal axons. The cells of central core of PACINIAN corpuscle may possibly secrete acetylcholin (Ach) when the corpuscles are stimulated, and Ach effects the terminal axons, thus the action potentialis initiated in the nerve,

BROWN (1948), however, denied the secretion of Ach in PACINIAN corpuscles. He dissected PACINIAN corpuscles from the mesentery of cats which had received 1 mg/kg of eserine, and they were extracted with 10 % trichloroacetic acid. The extract was tested on the cat's blood pressure and on leech muscle, and it was proved that the corpuscles contained no Ach.

The problem of Ach in PACINIAN corpuscles and the physiological action of non-specific ChE require further investigation.

2) According to ORSU (1953) et al., vagal afferent nerve fibers can be observed histologically in abdominal viscera.

Therefore, it is supposed that the afferent nerve from mesenteric PACINIAN corpuscles may be contained in the vagal nerve, and ChE activity in PACINIAN corpuscles can be considered to change after bilateral vagotomy.

But in the author's experiment, none of the corpuscles showed any change in ChE activity 3 weeks after the vagotomy, and moreover, 3 weeks after the rhizotomy on both sides between 8th and 11th thoracic dorsal roots, there was no alteration. As the ChE activity in PACINIAN corpuscle does not change after sectioning the posterior roots which surely terminate in it, so changes of ChE activity may not be expected after vagotomy, even if the vagal afferent nerve is distributed to the mesenteric PACINIAN corpuscles.

2 weeks after sectioning the sciatic and femoral nerves, ChE activity in the innervated PACINIAN corpuscles located in the subcutaneous tissue of the pad does not

change, while in the sectioned nerve itself ChE activity was reduced markedly.

From these results, it is considered that ChE activity in the central core of PACINIAN corpuscles has no relation to the degeneration of sensory nerves, and the cells of the central core are not neurogenic.

PEASE (1957) and HONDE (1959) also concluded by electron microscopic research that the cells of the central core are probably not SCHWANN's cell in origin.

COUTEAUX and NACHMANSOHN (1940), COUTEAUX (1942, 1947) observed biochemically, the ChE concentration at the motor end-plate decreased by less than one third within 3 to 4 weeks following the section of the motor nerve, and there remained stable for several months. Most of the enzyme presenting at the motor endplate is apparently localized in the post synaptic element, at most one third may be localized in the presynaptic membrane.

The change of ChE activity may occur only in the neurogenic part, supposing ChE in the central core of PACINIAN corpuscles may have same relation to that of motor end-plate.

3) Degeneration of the nerve became remarkable 3 or 4 days after nerve section, the axon swells first like a ball and the myelin sheath is destroyed in fragments, then SCHWANN's cells occupy these spaces. One week after sectioning, destruction of the myelin sheath is not marked, accordingly ChE activity in the nerve fiber is not appreciably reduced.

Concerning cholinacetylase activity in degenerated fibers, NACHMANSOHN (1946) stated that one third of the cholinacetylase activity was still present 3 days after sectioning the nerve, when conduction of nervous impulse had already disappeared.

2 weeks after the operation, SCHWANN's cells had proliferated and ChE activity had become very weak. The activity, therefore, may not be dependent upon SCHWANN's cells.

V. SUMMARY

1) Large part of ChE activity in the central core of PACINIAN corpuscles belongs to the non-specific type.

2) 3 weeks after posterior rhizotomy (Th 8-11) and bilateral vagotomy, ChE activity in mesenteric PACINIAN corpuscles does not show a remarkable change.

3) 1 week after the sectioning, ChE activity in the nerve does not change remarkably. But 2 or 3 weeks later, ChE activity became very faint.

4) ChE in PACINIAN corpuscles distributed in the subcutaneous tissue of cat's hairless pad does not change 3 weeks after the sectioning the sciatic and femoral nerves.

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REFERENCES

- 1) Brown, G. L. & Gray, J. A. B.: Some Effect of Nicotin like Substances and their Relation to Sensory Nerve. *J. Physiol.* **107**, 306, 1948.
- 2) Beckett, E. B., Bourne, G. H. & Montagna, W.: Histology and Cytochemistry of Human Skin. The Distribution of ChE in the Finger of the Embryo and the Adult. *J. Physiol.*

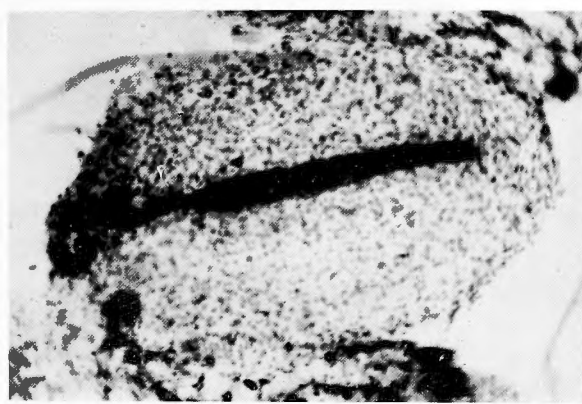


Fig. 1 ChE in the central core of mesenteric PACINIAN corpuscle, counterstained with hematoxylin. longitudinal section. ($\times 100$)

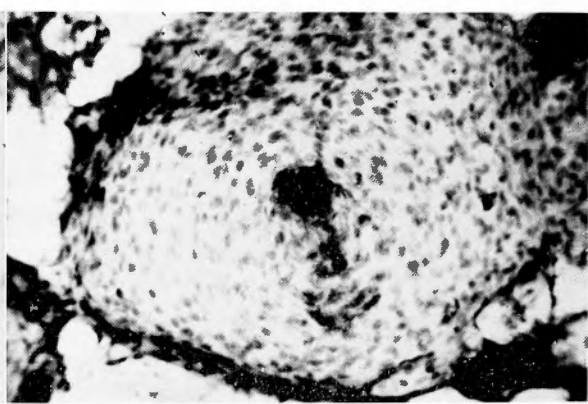


Fig. 2 Non-specific ChE in the central core of mesenteric PACINIAN corpuscle, counterstained with hematoxylin. transverse section ($\times 100$)

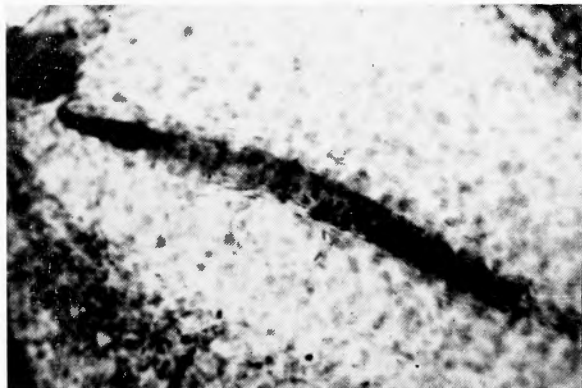


Fig. 3 ChE in mesenteric PACINIAN corpuscle 1 week after the posterior rhizotomy (Th8-11), counterstained with hematoxylin. longitudinal section. ($\times 100$)

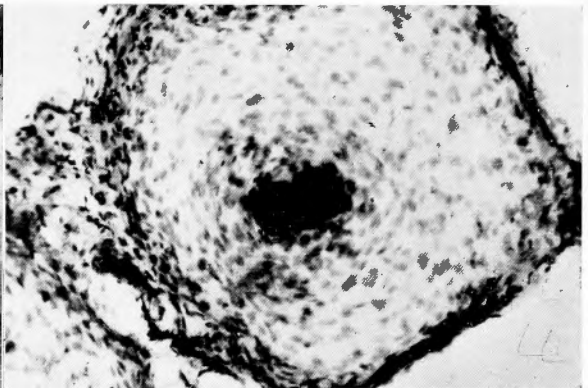


Fig. 4 ChE in mesenteric PACINIAN corpuscle 1 week after the posterior rhizotomy, counterstained with hematoxylin. transverse section. ($\times 100$)

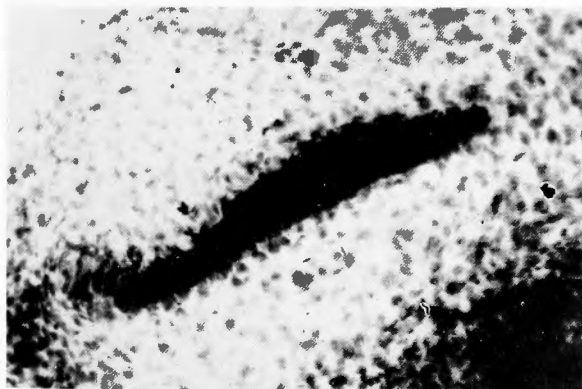


Fig. 5 ChE in mesenteric PACINIAN corpuscle 2 weeks after the posterior rhizotomy (Th8-11), counterstained with hematoxylin. longitudinal section. ($\times 100$)

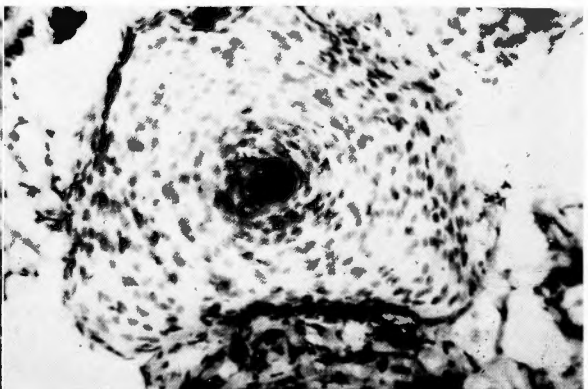


Fig. 6 ChE in mesenteric PACINIAN corpuscle 2 weeks after the posterior rhizotomy (Th8-11), counterstained with hematoxylin. transverse section. ($\times 100$)

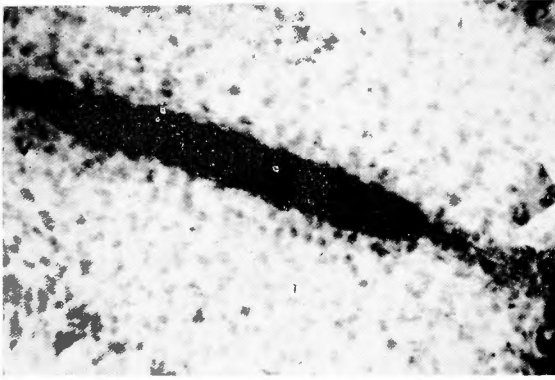


Fig. 7 ChE in mesenteric PACINIAN corpuscle 3 weeks after the posterior rhizotomy (Th8-11), counterstained with hematoxylin. longitudinal section. (x100)

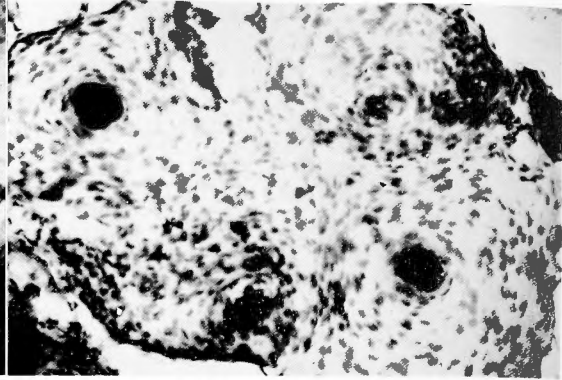


Fig. 8 ChE in mesenteric PACINIAN corpuscle 3 weeks after the posterior rhizotomy (Th8-11), counterstained with hematoxylin. transverse section. (x100)

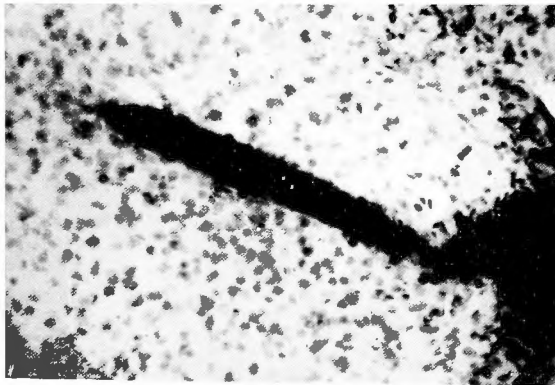


Fig. 9 Non-sp. ChE in mesenteric PACINIAN corpuscle 3 weeks after the vagotomy, counterstained with hematoxylin. longitudinal section. (x100)

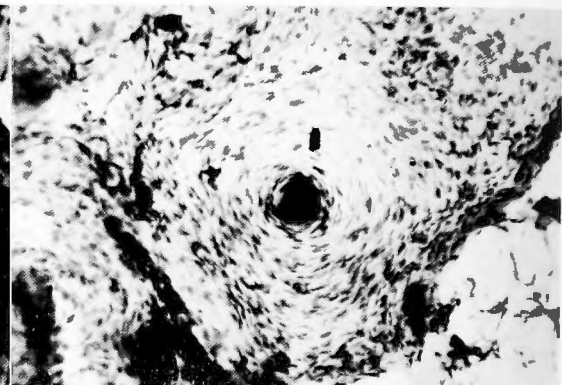


Fig. 10 ChE in mesenteric PACINIAN corpuscle 3 weeks after the bilateral vagotomy, counterstained with hematoxylin. transverse section. (x100)

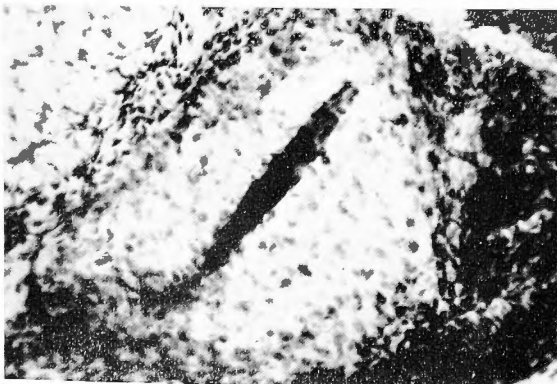


Fig. 11 ChE in PACINIAN corpuscle in the subcutaneous tissue of the pad 3 weeks after sectioning the sciatic and femoral nerves, counterstained with hematoxylin. longitudinal section. (x100)

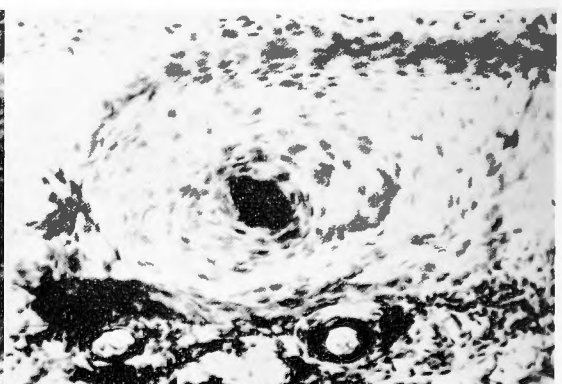


Fig. 12 ChE in PACINIAN corpuscle in the subcutaneous tissue of the pad 3 weeks after sectioning the sciatic and femoral nerves, counterstained with hematoxylin. transverse section. (x100)

Fig. 13

a) ChE in normal sciatic nerve.
($\times 100$)

b) ChE in sciatic nerve, 1 week
after sectioning the nerve.
ChE activity is not yet reduced.
($\times 100$)

c) ChE in sciatic nerve, 3 weeks
after sectioning the nerve.
ChE activity is weak, and SCH-
WANN'S cells have proliferated.
Counterstained with he-
matoxylin. ($\times 100$)

134, 202, 1956.

- 3) Couteaux, R. & Nachmansohn, D. : Change of ChE at End-plates of voluntary Muscle following Section of Sciatic Nerve. *Proc. Soc. exp. Biol., N. Y.* **43**, 177, 1940. Cited by Fulton (7)
- 4) Couteaux, R. : La ChE des plaques motrices après section du nerf moteur. *Bull. biol.* **76**, 14, 1942. Cited by Fulton (7)
- 5) Couteaux, R. : Contribution à l'étude de la synapse myoneurale. *Rev. Canad. Biol.* **6**, 563, 1947. Cited by Fulton (7)
- 6) Douglas, W. W. & Gray, J.A.B. : The Excitant Action of Ach and other Substances on Cutaneous Sensory Pathway and its Prevention by Hexamethonium and d-Tubocurarine. *J. Physiol.*, **119**, 118, 1953.
- 7) Fulton, J. F. : *Physiology of The Nervous System*. Oxford University Press. 1949.
- 8) Gray, J. A. B. & Sato, M. : Properties of the Réceptor Potential in Pacinian Corpuscle. *J. Physiol.*, **122**, 610, 1953.
- 9) Hellman, K. : ChE and Amine Oxidase in the Skin ; A Histochemical Investigation. *J. Physiol.*, **129**, 454, 1955.
- *10) Honde, S. : Electron Microscopy of the Development of Pacinian Corpuscle. *Arch. Jap. Chir.* (in press)
- 11) Koelle, G. B. & Friedenwald, J. S. : A Histochemical Method for Localizing ChE Activity. *Proc. Soc. exp. Biol. & Med.*, **70**, 617, 1949.
- 12) Koelle, G. B. : The Histochemical Differentiation of Typus of ChE and their Localizations in Tissue of the Cat. *J. Pharm. Exp. Therap.*, **100**, 158, 1950.
- 13) Koelle, G. B. : The Elimination of Enzymatic Diffusion Artifacts in the Histochemical Localization of ChE and a Survey of their Cellular Distributions. *J. Pharm. Exp. Therap.*, **103**, 153, 1951.
- 14) Koelle, G. B. : The Histochemical Identification of Acetyl-ChE in Cholinergic, Adrenergic and Sensory Neurons. *Ibid.*, **114**, 167, 1955.
- 15) Nachmansohn, D., John, H. M. & Berman, M. : Studies on Choline Acetylase. II The Formation of Ach in the Nerve Axon. *J. Biol. Chem.*, **163**, 475, 1946. Cited by Fulton (7)
- *16) Okinaka, S. & Yoshikawa, M. : Recent Advance of Studies on ChE. *Nisshin Igaku*, **37**, 1, 1950.
- *17) Okinaka, S. & Yoshikawa, M. : Recent Advance of Studies on Ach and ChE. *Nisshin Igaku*, **40**, 371, 1953.
- 18) Otsu, A. : A Histological Study of Sensory Nerve Ending in Alimentary Canal of the Human Beings and Dogs. *Acta Sch. Med. Univ. Kyoto Jap.*, **31**, 103, 1953.
- 19) Pease, D. C. & Quilliam, T. A. : Electron Microscopy of the Pacinian Corpuscle. *J. Biophysic. & Biochem. Cytol.*, **3**, 331, 1957.
- 20) Seehan, D. : The Cell Station of the Vater-Pacinian Corpuscle in Retroperitoneal Tissue. An Afferent Peripheral Pathway in the Sympathetic. *Brain*, **55**, 493, 1932.
- 21) Suzuki, K. : Histochemical Studies on ChE in Pacinian Corpuscles. *Arch. Jap. Chir.*, **27**, 1055, 1958.
- *22) Seto, H. : Studies on the Sensory Innervation. *Igaku Shoin*, 228, 1957.
- *23) Toyoda, M. : Histochemistry of ChE. Report 1. *Nisshin Igaku*, **40**, 559, 1953.
- *24) Toyoda, M. : Histochemistry of ChE. Report 3. *Ibid.*, **42**, 243, 1954.
- *25) Uono, M. : Nervous System and ChE. *Sōgō Rinsyo*, **6**, 189, 1957.
- *26) Uono, M. : Enzymes related to Chemical transmitter of Autonomic Nervous System. *Nihon Rinsho*, **16**, 943, 1958.

* Written in Japanese.

和 文 抄 録

パチニー氏小体に於けるコリンエステラーゼの
組織化学的研究 第2報

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鈴 木 克 義

Koelle (1951) の方法を用いて、猫の腸間膜及び後肢臍部の皮下に存在するパチニー氏小体のコリンエステラーゼについて、組織化学的に検索し、次の結論を得た。

1. パチニー氏小体の内棍部に存在するコリンエステラーゼは、その大部分が非特異性のコリンエステラーゼである。

2. 後根切断 (Th8-11), 或は両側迷走神経の切断3週後では腸間膜パチニー氏小体のコリンエステラー

ゼー活性に著明な変化を認めなかつた。

3. 坐骨神経は、切断1週後では、コリンエステラーゼの活性にあまり変化を認めないが、2～3週後では著明な活性の低下を認めた。

4. 坐骨神経及び股神経切断3週後には、上述のように神経自身に著明なコリンエステラーゼの減少を認めたのに拘らず、その支配下の臍部皮下に存在するパチニー氏小体では著明な変化を認めなかつた。