The Ultrastructure of Lafora Body

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(Received December 3, 1966)

INTRODUCTION.

Myoclonus epilepsy (Lafora type) was proved differently from the other diseases with myoclonic jerk, by Hodskins et al.[13] and Weingarten.[49] Namba et al.[24 25] reported as the clinical features of the myoclonus epilepsy, about the epileptic seizure, personality change before the onset of symptomes and the age of death which occured within the period of 17 and 24 years. Namba and Ota[31] reported also the serial investigation of electroencephalogram about the family of myoclonus epilepsy and revealed in a sibling (sixth-horn) of the myoclonus patients who had appeared healthy and well entirely, normal electroencephalogram on 5 years of age, 3 per second spike and wave seizure pattern without the clinical signs on 7 years of age and suggested that the electroencephalogram could take place in the elucidation of the familial disposition or early recognition of the patients.

Since 1925, when Lafora et al.[20] suggested that the etiology of this disease might be a result of metabolic defect in the central nervous system, the hypothesis has been agreed by many authors. So, histological research for this disease tends to aim histochemical analysis of Lafora body as attempted by Westphal[50], Ostertag[33], Harriman et al.[11], Oyake et al.[14], Seitelberger et al.[42] and Namba[28]. The recent progress in this field developed to neurochemical analysis and electron microscopic study. The former includes clinico-biochemical research studied by Harriman et al.[11], Namba et al.[29] and van Heycop ten Ham et al.[48] and the chemical analysis of the brain studied by Edgar,[6] Suzuki[45] and Yokoi[51], the latter includes the researches of Namba,[28] Seitelberger et al.[42], and Schwarz et al.[40] utilizing autopsied specimen. Electron microscopic study about biopsied brain tissue was reported by Janeway et al.[16] on the first occasion, followed by the report of Namba and Ota[32] concerning the comparison of electron microscopic findings of Lafora body between biopsied and autopsied materials. Both reports, however, are not sufficient to clarify the

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* To Prof. Gerd Peters, this paper is dedicated in celebration of his 61st birthday.

ultrastructure of Lafora body completely.

This report attempts to elucidate further details of the ultrastructure of Lafora body and its relation with other cellular components so as to clarify how Lafora body has been made.

MATERIAL AND METHOD.

The first born of the sibling which included biopsied patient, was succumbed to myoclonus epilepsy of Lafora type at the age of 19. Autopsy findings were as follows: numerous Lafora body in the central nervous system; characteristic deposit in the parenchymal cells of the liver and heart muscle. The second born had the same disease, died of drowning at the age of 15. The autopsy findings were consistent with that of the first born. The sixth born, 9-year-old male, as stated in the introduction, developed epileptic seizure pattern at the age of the 7th years.

This patient, the third born, is 16-year-old female. Personality change has developed preceding the fiirst grand mal attack at the 9 years of age, followed by the myoclonic jerks 6 months later. Cerebellar ataxia at 15 years of age. The aggravation and aleviation of these symptomes alternated until 15 years and 6 months, when this study was intended, there were disarthria, apathy, generalized convulsions 1 to 2 times a month, intermittent muscle twitchings in the facial musculatures and in the extrimities, hypotonia on extremities, the joint ankylosis in flexed position. The body temperature excursed near 38 degree centigrade. Nasal tube feeding was necessary due to the incapability of voluntary swallowing and the decubiti were increasing in size and number.

Craniotomy for cerebral cortical biopsy was performed at 15 years and 9 months of age, under the general anesthesia induced by pentothal and nitrous oxide. Tissue block was excised from rostral portion of the cortex of Brodmann's area 9, under antihemorrhagic maneuver. The material was devided into 3 parts. The first was examined by phase contrast microscopy pressed between the slide and cover glasses. The second was used to histochemical study. The third was examined by the electron microscope.

For the electron microscopic study, some pieces of the block were fixed with 1 per cent osmium tetroxide solution buffered by phosphate for 30 minutes, and the others were fixed with buffered 6 per cent glutaraldehyde solution for 30 minutes, followed by refixation by 1 per cent osmium tetroxide for 30 minutes, dehydrated by ascending series of aceton, embedded in Epon 812, ultrathinsectioned by Porter-Blum ultramicrotome MT-I, stained by lead citrate, and photographed by electron microscope JEM-5HS-560, manufactured by Nippon Denshi K.K.

FINDINGS.

Descriptions are devided into : 1) cortex without Lafora body, 2) Lafora body in the neuronal perikarya, 3) small sized Lafora body, 4) structures simulate to Lafora body.

1) Cortex witout Lafora body.

The smooth and rough surfaced endoplasmic reticulum (sER and rER), ribosome, Golgi apparatus, mitochondria and neurofilaments appear normal, however cystic dilatation of mitochondria with electron lucent matrix are observed occasionally. Plasma membrane of neuropil, synaptic visicle, synapses appear normal in contour and amount. Some elongated vesicles are encountered at times in the dendritic process. Normal myelin sheaths (Fig. 1.)

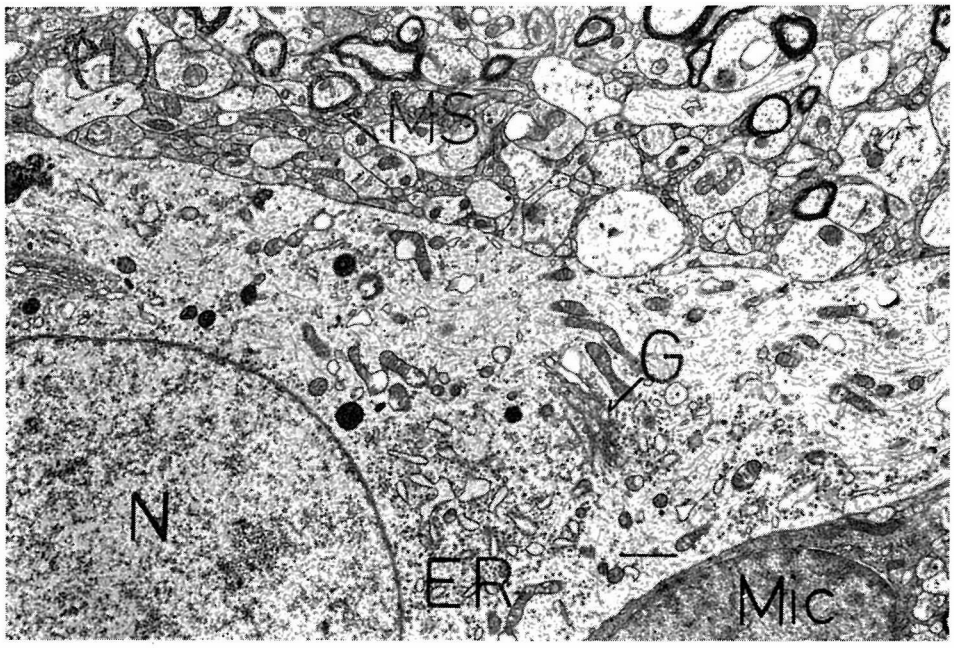

Fig. 1) Normal cerebral cortex. Nerve cell, microglial cell and cell processes. X. 72,100

2) Lafora body in the Neuronal perikarya.

Perikaryal inclusions are generally large or medium sized. The external and the internal layers are not so clearly demarcated in electronmicrograph as in the autopsied material but also seen in the biopsied one (Fig. 2.) This difference in the external and the internal layer of the body proved to be the characteristic finding of the body, because unfixed and unstained specimen also show same findings by phase contrast microscopy (Fig. 3.) Many bodies compress the plasma membrane and generally no cavities are found, at times, however, there are cy-

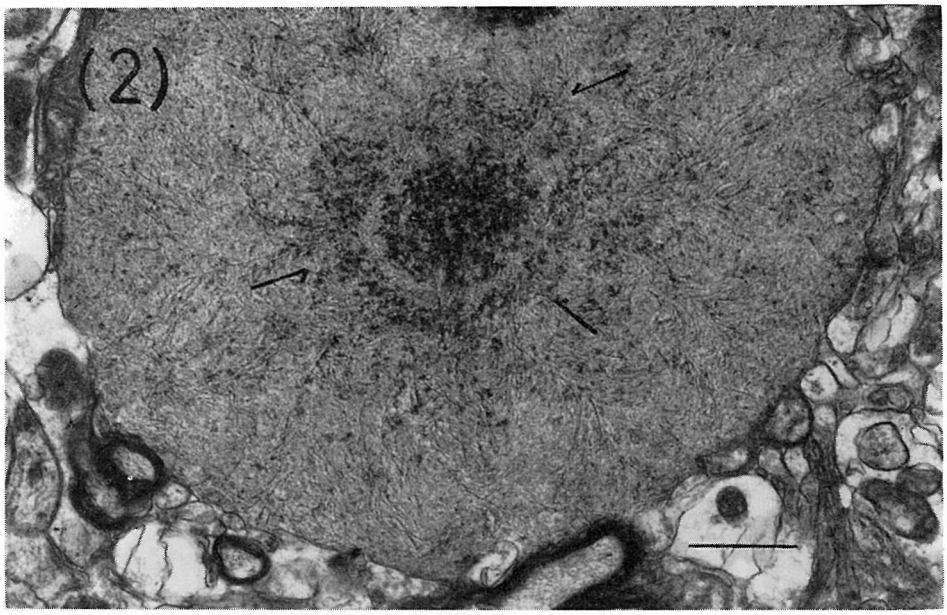

Fig. 2) The layered structure of Lafora body. X. 13,600

stic mitochondria, Golgi apparatus or filamentous structures bitween them (Fig. 4.) Outer membrane of the nuclear envelope of nerve cells distended occasionally to the perikaryon in largely vacuolated fashion (Fig. 5.) The large or medium sized body has no limiting membrane proper, nor any direct contact with the cell and the nuclear membrane. External layer of Lafora body in the neuronal perikarya contains characteristic lamellar structure, within it, there are enlarged and vacuolated ER, dilated mitochondria with vague membrane and cristae and electron lucent matrix, or the electron dense matrix with vague membrane and cristae, ribosome-like granules, dense bodies and possibly neurofilaments. Interspersed cytoplasmic organelles are more degenerated in the internal layer than in the external one. The internal layer of the body is dense in electron micrograph, at times lighter, however, with scarce cytoplasmic organelles and appears homogenously (Fig. 6.) Relatively large portion of Lafora body is generally speaking occupied with the cytoplasmic organelles. Characteristic structure seen in the external layer of the body is short branching lamellar structure. The lamella consists of double fila measures about 50 to 100 Å thick with opaque interspace. On occasion, they fuse into single dense filament and in another occasion, they appear beads-like. The lamella terminate by the blind canal or remain opened. Lamella seen in the external layer frequently show beads-like appearance, attached by the fine granular substance on the surface. Interspace of the lamella filled with less dense, fine granular or amorphous claudy substance, which is not ide-

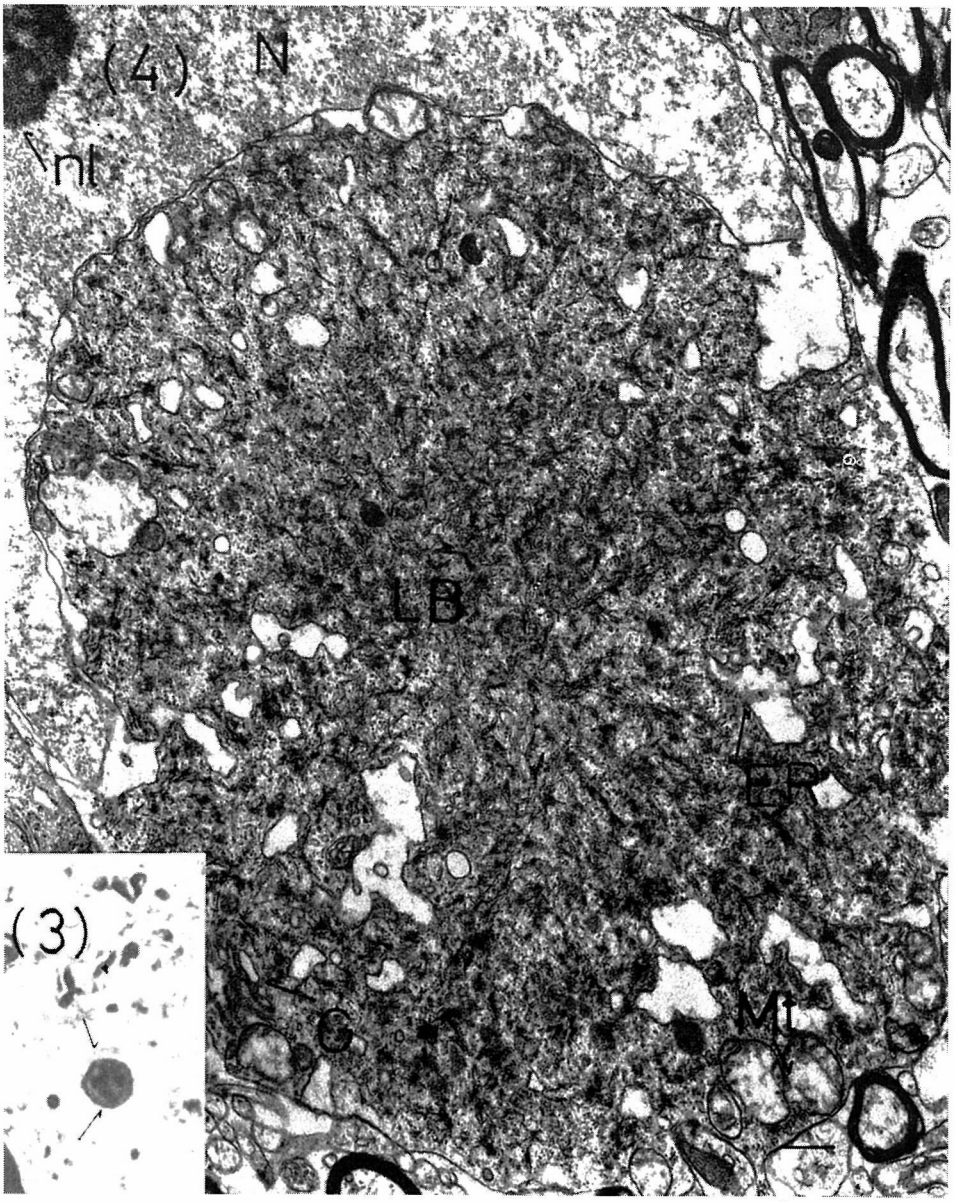

Fig. 3) Phase contrast microscopic figure of Lafora body. X 370
Fig. 4) Cytoplasmic organelles included in the large sized body. X 7,340

ntified as profiles of the transected lamellae. Short branching lamellae appear, at times, rigidified and arranged in stellate or bundle form and at the confluent region of the lamellae, there are scattered dense granules. These lamellae generally has the tendency of radiate arrangement from the center of the body to the periphery. Dense granules show higher electron density and larger several

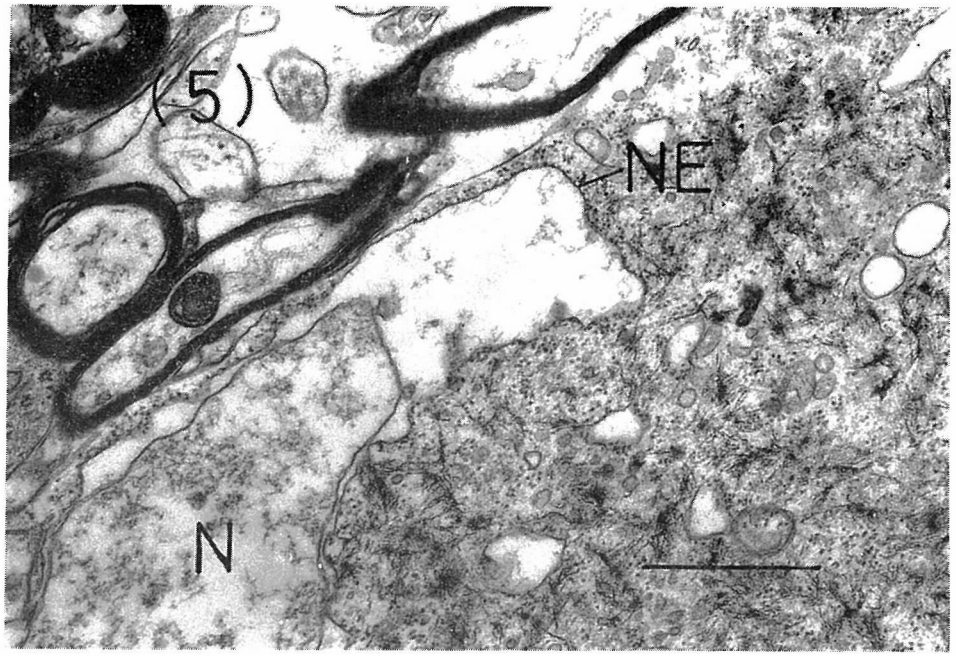

Fig. 5) Distended nuclear envelope. X 23,000

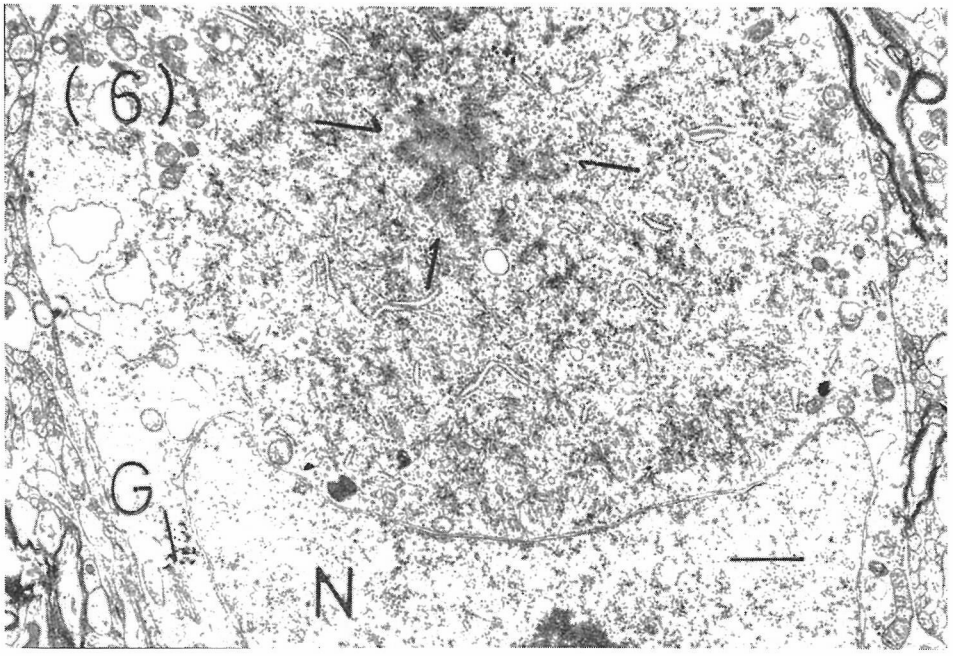

Fig. 6) Dense, homogenous internal layer. X 9,600

times than ribosomes which seen in and around Lafora body. These simulate glycogen-like particle in the astrocytic process although they are ill defined.

Lamellae in the internal layer are not clearly defined than in the external layer and tend to lose their characteristic lamellar structure, and degenerate to denser and granular appearance (Fig. 7.) The lamellae are occasionally concentrated irregularly and closely. In the internal layer, glycogen-like granules are also seen as in the external one but generally the outer limits are more vague. As in the Fig. 2, however, glycogen-like granules may clearly be seen. Central zone of the internal layer are as seen in Fig. 6, and Fig. 8, more homogenous, denser, irregularly shaped in stellate appearance and the lamellar structure disappear or show the lower electron density.

3) Small sized Lafora body.

Its location is mainly in the cell process. As shown in Fig. 9, the location is in the postsynaptic ending and has no relation with the synaptic apparatus. In the small sized body, no cytoplasmic organelles or dense bodies are intermingled togather and the structure is simpler. Lamellae are relatively smooth and thick, and show scarce tendency to branching, bundle forming or stellate arrangement. There is also no lmiting membrane around the small siized Lafora body. In other small sized body, there are elongated vesicles between the body and the cytomembrane. In some portion of the vesicle, the electron density of the wall may

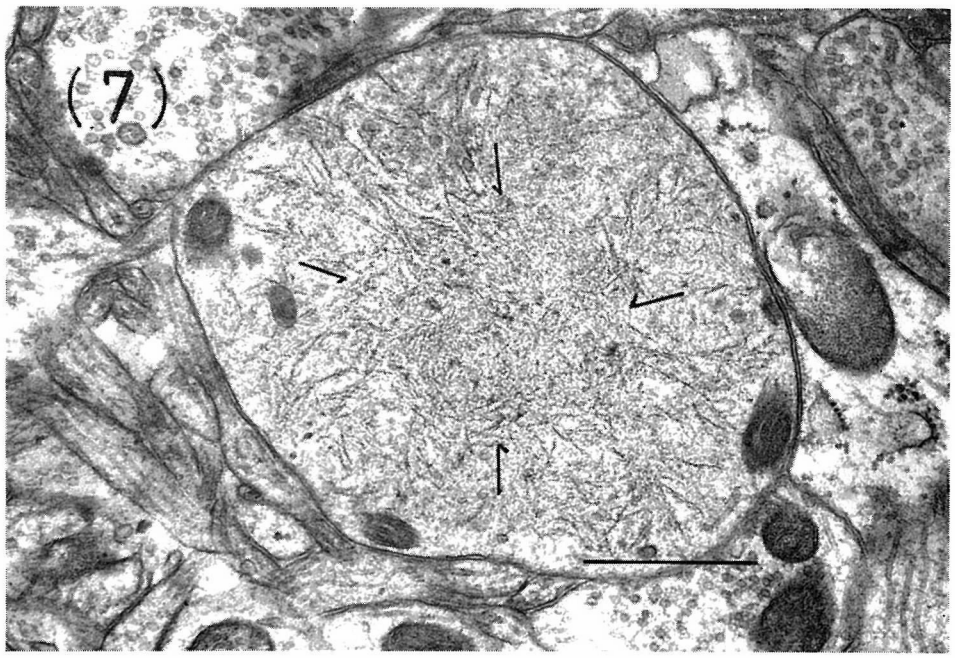

Fig. 7) Granular internal layer. X 22,000

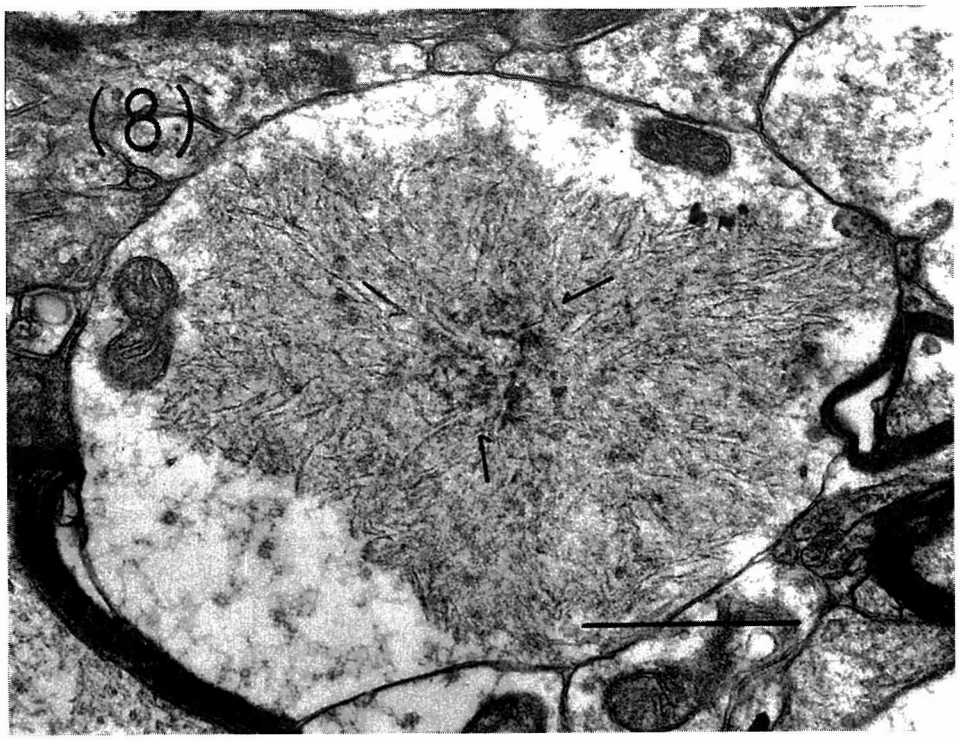

Fig. 8) Dense internal layer. X 27,100

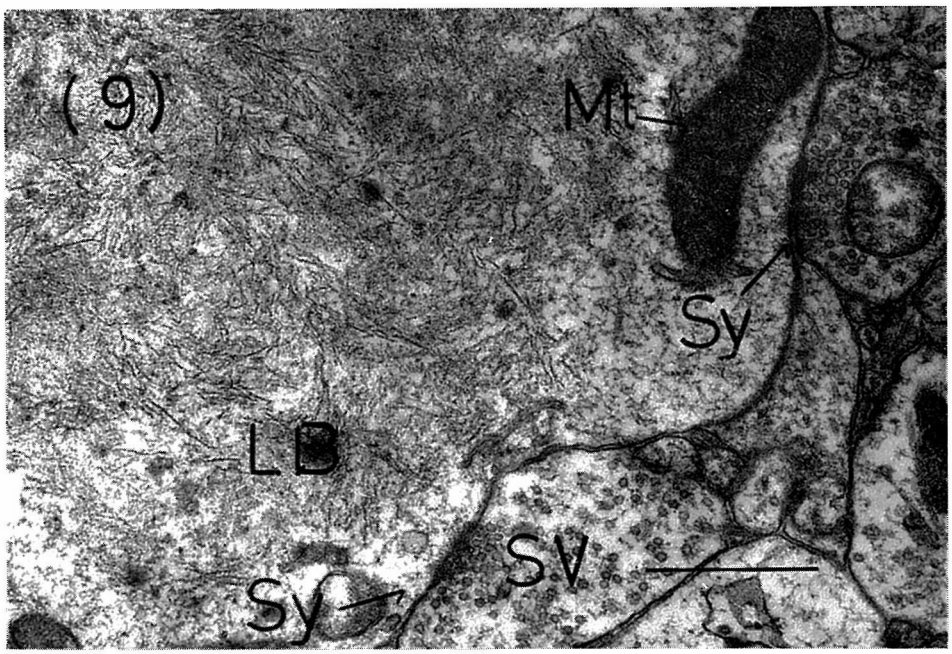

Fig. 9) Lafora body located in the postsynaps. X 22,000

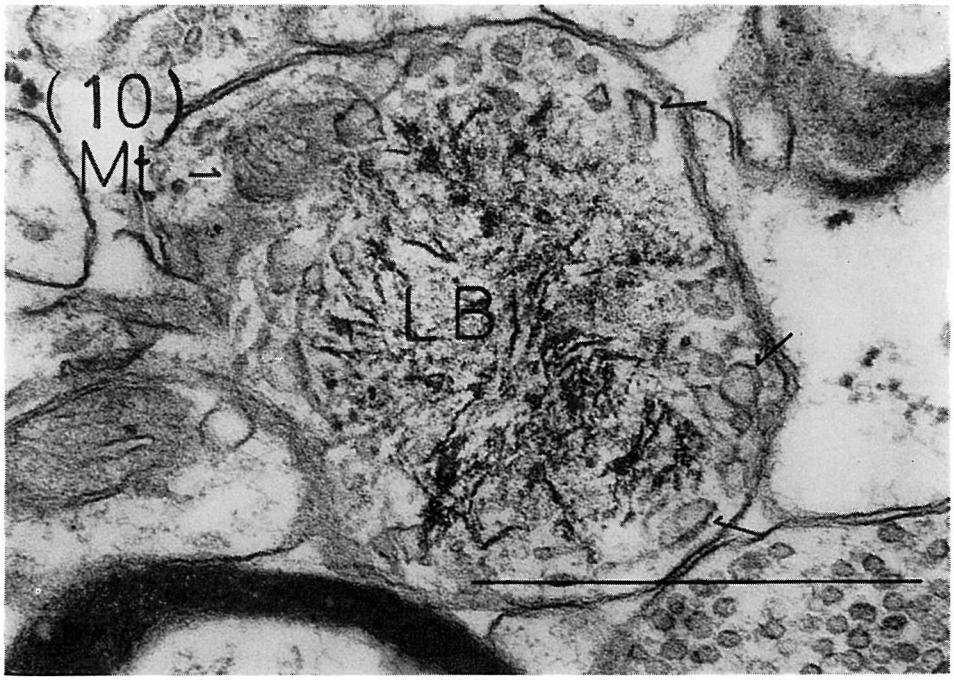

Fig. 10) Elongated vesicles surrounding Lafora body. X 57,700

be high, and is narrowed as if they were rigidified. In the rigid area, bilateral walls become closer, and simulate to peculiar lamella of Lafora body. Other elongated vesicles show thickening of the wall and become less dense (Fig. 10.) The vesicle may possibly be in transferring stage of the two vesicles, the one which located freely in the area between the body and the plasma membrane and the other which located in contact with the plasma membrane. In the latter, plasma membrane protrudes partially into the cell process verrucously and mushroom-like, then the narrowing of the base and detatching from the membrane may possibly take place as in pynocytotic process. It seems very early stage of the above process. Distended area of plasma membrane contains homogenous, less dense substance possibly from the intercellular space (Fig. 11.) Free vesicle or elongated vesicle may contain, as stated above, substance from the intercellular space and ought to be differentiated from ER. These vesicles occasionally show almost consistent appearance with the synaptic vesicle. From the breath of the wall of vesicles, however, size of the vesicle and location that the vesicle situated in postsynaps, it is differentiated from synaptic vesicle. When Lafora body located in the postsynaptic terminals, as seen in the Fig. 9, subsynaptic weband interstitial space are less marked. Lafora bodies are encountered in the myelinated axon on rare occasion. (Fig. 12.)

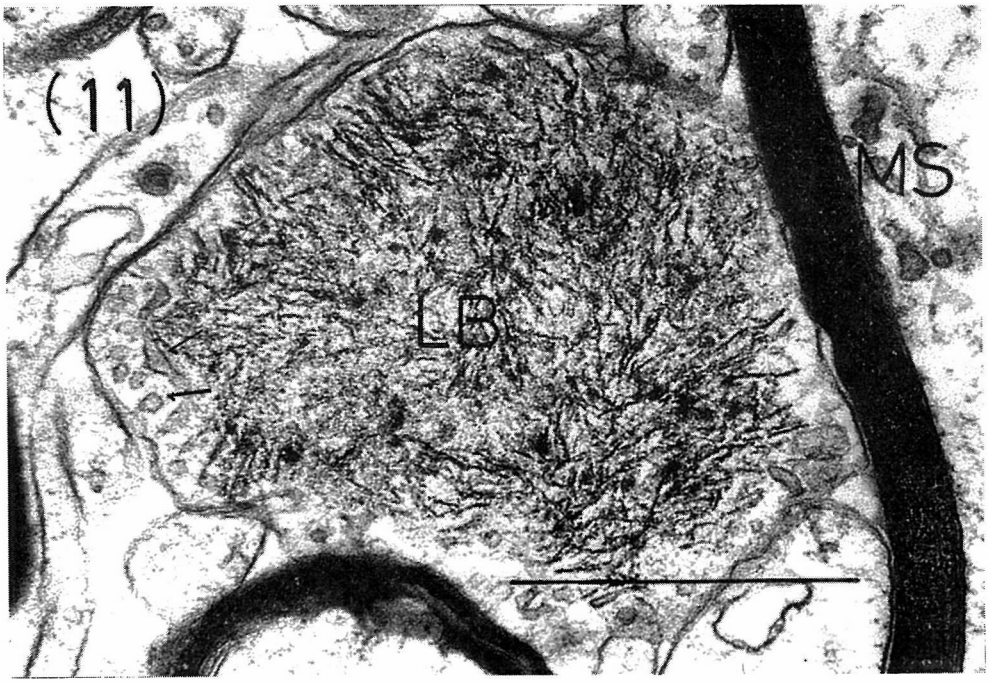

Fig. 11) Structures around the small sized body. X 44,700

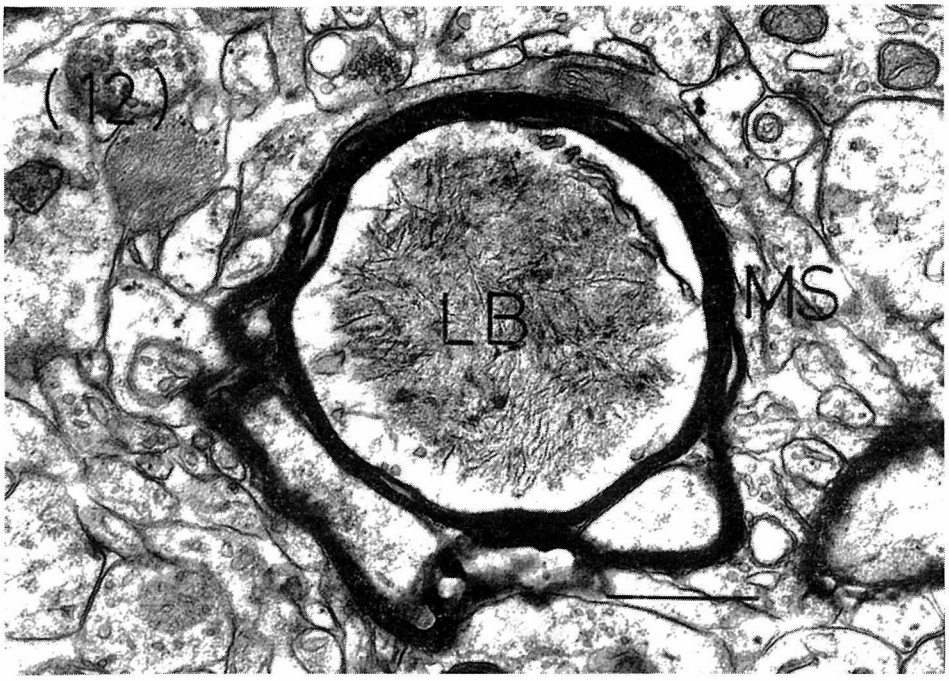

Fig. 12) Lafora body in the myelinated fiber. X 21,900

4) Structures show similarity to Lafora body.

a) Mitochondria.

Except for mitochondria surrounding Lafora body, there are mitochondria with ill defined wall with exposed cristae mitochondriales and matrix in the neuronal perikaryon (Fig. 13.)

b) Neurofilament.

Neurofilament in the neuronal perikaryon runs randomly, but in the cell process, they are arranged relatively regular and pararelly. Neurofilament has smooth surface and its transected profil is uniform, measures about 100 to 200 Å in the diameter which definitely differs from lamellar structure of Lafora body. (Fig. 14.)

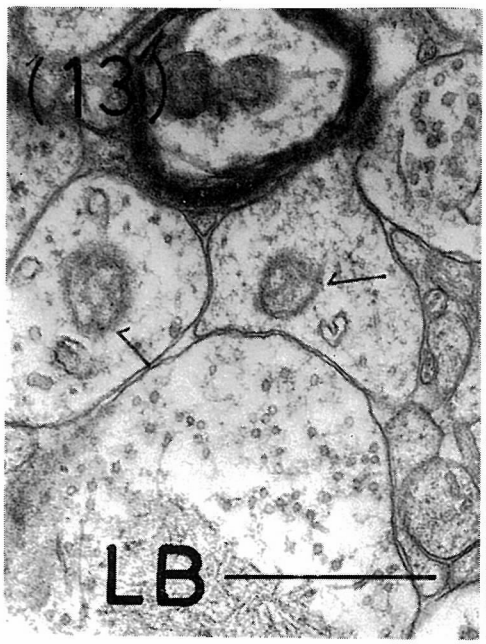

Fig. 13) Mitochondria. X 27,500

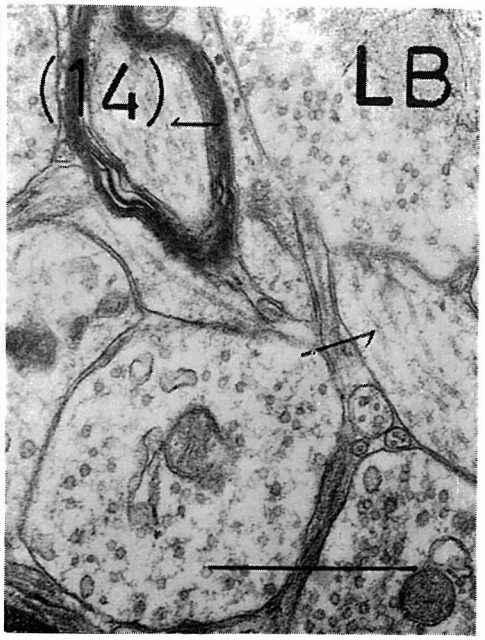

Fig. 14) Neurofilament. X 27,500

Key to Labeling on All Figures:
ER, endoplasmic reticulum
G, Golgi apparatus
LB, Lafora body
Mic, microglial cell
Ms, myelin sheath
Mt, mitochondria
N, nucleus
NE, nuclear envelope
ni, nucleolus
SV, synaptic vesicle
SY, synaps

COMMENT AND CONCLUSION.

Namba and Ota [32] have stated that in their comparative study of Lafora body in autopsied material and in biopsied one, in both material, characteristic lamellar structure are relatively preserved and indicated that autopsied material could possibly be utilized to some sort of electron microscopical study of Lafora body in future. Nevertheless, the biopsy is important method to investigate the relation of the body and spared cellular organelles.

According to Janeway et al. [16], the ultrastructure of Lafora body taken by biopsy disclosed that it consisted of fine fibrils with scattered dense clumps. When the nerve cell include the largest inclusion body, cytoplasmic organelles are almost completely disappeared. The inclusion body has no limiting membrane and located also in the cell process, including axon.

Namba [28] stated in the preceding report that in order to know how the body was made, it might become the important way to clarify the location of Lafora body in the neural tissue. In this study, as Janeway stated, intraaxonal localization was found. But these are so rarely encountered that Lafora body mainly arise in the cell position other than axon.

The fact that mentioned above suggests that Lafora body has relation with some sort of cytoplasmic organelles and also suggests that have no or scarce relation with mitochondria, synaptic vesicles.

For further details about the relation of ultrastructure of Lafora body, Namba [28] stated in the light microscopy, with formol or formol alcohol fixed preparation, revealed characteristic layered structure and radiate appearance. In unfixed preparation with phase contrast microscope, however, showed only layered structure but the radiate appearance were vaguely demonstrable. In other word, layered structure of the body is the characteristic nature and suggest that the radiate appearance may possibly be an artifacts. The findings responsible to this light microscopic study, large sized Lafora body revealed, by the electron microsocpe utilizing the biopsied material, that the body is made by mixing up of lamellar structure with cytoplasmic organelles. Through this mixing, on the one hand, Lafora body may increase in size and amount, become medium or large sized, on the other, may influence the inner structure of the body secondarily.

Possibly by the difference of condition at the fixation, morphological difference from randomly mixed state, showed in this report, to the radiate structure may appear as the result of separation of Lafora body proper with cell body.

As already warned by Namba, to evaluate the result of histochemical or chemical analysis of the medium or large sized Lafora body, the fact that cytoplasmic organelles are mixed in the body should be meticulously accounted for. As Namba reported formerly about the autopsied specimen, it was clarified more dicidedly in this report that the internal layer of Lafora body may include

moderately variable components from the degenerated external layer to concentrateddense granules.

It may be thought that small sized Lafora body is juvenile form and almost purely consisted of characteristic structure and content. Lamella of small sized body is smooth, shows less branching tendency. Opposing that the external layer of medium or large sized Lafora body consists of possibly transected and rigidified short branching lamellae and in the internal layer become granular in character. These changes of lamella appearing associated with the enlarging of Lafora body, may result the senile change or disturbance in catabolic phenomenon and may reflect the chemical composition of the membranous structure. The fact that dense granule attached to lamella which simulates glycogen-like particle in the process of astrocyte are seen rarely in the small sized body and frequently in the medium or the large sized body may suggest the lamella sustaining metabolic anomaly, conjugated with chemical change.

Concerning the relation of Lafora body with cytoplasmic organelle, we are interested in vesicle than mitochondria. Especially elongated vesicles which are located freely from plasma membrane, surrounding the small sized body in the cell process may be important. Membrane of these vesicles may show the nature of unit membrane, swollon partially and simulates the lamella of Lafora body.

Namba [29] showed the possibility that in Lafora body, there were phospholipids, mucopolysaccharides, small amount of protein and iron. These facts may support our hypothesis about morphology above mentioned and the origin and developement of the lamella of Lafora body. In the vesicles we showed, its contents seem to be taken into the vesicle from the extracellular space. Hess [12] suggested the existense of mucoprotein in the extracellular space, Sjöstrand [43] proved lipids in it. Robertson [38] stated that it consisted of polysaccharides and Porter [36] suggested existence of mucopolysaccharide. These are interesting to us, however, that elongated vesicles above mentioned are not always found in the neighborhood of Lafora body. Some are located not related to Lafora body. But these facts may possibly be interpreted considering that Lafora body may be transported following the plasma flow. In Lafora body, except for lamellar structure, dense, amorphous or fine granular substance fill the lamella's interspace. Since some of the lamella seem to have been conjugated with filamentous substance around Lafora body, it cannot be neglected that this substance has some role in the process of Lafora body formation.

Dense granule attached to lamella has been found moderately in the medium or large sized one. So this granule may be the by-product in catabolizing mechanism of the lamella. As already stated, both structures do not play any fundamental role in Lafora body formation.

Table
Different Features of Metabolic Diseases

Diseases	Structure	Chemical Composition	Location
Lafora's Disease	short branching double filament. (100 A in diameter)	mucopolysaccharide, Phospholipids, protein, iron,. (congo red (−))	neuronal cytoplasm, dendrite and astrocyte
Corpora Amylacea (Ramsey)	electron dense fibrillae, electrontranslucent cleft and glycogen particle.	highly polymerized mucopolysaccharide, (Diezel) polysaccharide.	process of astrocyte.
Amyloid (Diezel)	filamentous feltwork. (100 Å in diameter) (Kidd)	acid or neutral mucopolysaccharide, lipoprotein.(Diezel) (congo red (+))	heart, kidney, spleen etc. extracellular amyloid.
Familial Mediteranean Fever	beased-like fine filamentous material. (200-300 Å in diameter) (Cohen)	amyloid, glycoprotein plus sulphated compound. (Kennedy)	capillary, kidney.
Alzheimer's Disease (Palay et al) (Suzuki) (Kidd)	fine, long threads. (60 to 100 Å in diameter) irregular filamentous tubules. (two stranded helices) (Kidd)	phrenosin and kerasin striking increased. (Suzuki)	neuronal cytoplasm or postsynaptic process.
Experimental production of neurofibrillary degeneration (Terry et al) (Pena)	filament fasciculus. (50 Å in diameter)	acid phosphatase activity absent (Terry) acid polysaccharide (hyaluronic acid, chondroitin sulfate) protein fibril. (Klatzo)	perikaryon
Gargoylism (Aleu) (Lagnoff)	irregular clear vacuole. zebra body.	chondroitin sulfate B and heparin sulfate. (soluble mucopolysaccharide) (Lagnoff) ganglioside, cholesterol, phospholipids, mucopolysaccharide. (formol soluble)	perithelial cytoplasm, neuronal cytoplasm, liver, spleen, reticuloendothelial system.
Tay-Sachs's Disease	lamellated membranous cytoplasmic body. (spiral or pararellar arranged three layered unit membranes) (Samuel et al)	ganglioside (monosialoganglioside) (Gonatas) cholesterol, protein, phospholipids, amino scids, ganglioside. (Samuel et al) globoside. (Yamakawa and Ionse)	neuronal cytoplasm, mitochondria.
Late Infantile Systemic Lipidosis (Ledeen)	membranous cytoplasmic body.	ganglioside. (galactoseglucose-galactosaminsialic acid) (Ledeen) phospholipids, cholesterol, protein, amino acid, peptide. (Gonatas)	neuronal cytoplasm, mitochondria.
Nieman-Pick's Disease	lamellar structure.	sphingolipids.	neuronal cytoplasm
Unusual Neocortical Presynaptic terminal (Gonatas)	branching membranous structure. (150 to 100 A in diameter)		presynaptic terminal.
Vitamin E defficient Rat (Lampert)	axonal swelling, numerous neurofilaments and vesicular profiles, branching tubular profile and electron dense body.		axon terminals inthe gracil and cuneate nuclei.
Gaucher's Disease (Philippart)	filamentous body.	ceramide monohexoside. (ceramide dihexoside)	reticuloendothelial system.

Jakob-Creutzfeldt's Diseasse (Gonatas et al)	densely packed, dolid fibril. (70 to 90 Å thick) electron dense body, membranous osmiophilic body.		astrocyte, abnormal synaps, presynaptic ending.

The table shows comparison of structure, chemical composition and location of the structure similar to Lafora body. In this table, most similar to Lafora body is the electron micrograph of corpora amylacea reported by Ramsey [37]. The ultrastructure of corpora amylacea is composed of dense, short filaments, dense lines, matrix and glycogen particles. She stated that occasionally cytoplasmic elements were included and that as difference between Lafora body and corpora amylacea, Lafora body might originate in the perikaryal cytoplasm of the neuron, and we also think that the topographical difference is important. Both bodies are at least, in the medium sized, show very similar appearance in lamellar structure, however, concerning the problem whether from the small or the medium to the large sized body, whether the structure and the composition of Lafora body and amyloid body are consistent or not, further comparative study will be necessary for the matter.

The filament of general amyloidosis [5] is smoother and tends to be short branching than that of Lafora body and differs in the site of origin. Fine filamentous material seen in familial mediteranean fever [2] differs from Lafora body in the site of predilection and the diameter of filament. In Alzheimer's disease, [18,44] filaments run pararelly and the surface of the filaments is smoother than the lamella of Lafora body, and cannot be found in thhe postsynaptic process or myelinated fiber. Experimental neurofibrillary degeneration [46] differs from Lafora body because the filaments run in fasciculi and the transected face of the filament has the hollow in its center. Inclusion body found in Gargoylism[1], Tay-Sachs disease [39], late infantile systemic lipidosiis [23] are decidedly apart from Lafora's inclusion. In Nieman-Pick's disease, the inclusions are different from Lafora body in general appearance, but it is interesting that the basic structure is consisted of lamella. Unusual neocortical presynaptic terminals[7], show also branching membranous structure, the location and thickness of structure does not, however, consistent with Lafora body. Filaments seen in the vitamin E defficient rat [22], differ from lamella of Lafora body becouse it runs longitudinally and continuously to the axon. The neuroaxonal dystrophy [41], seen in the late infantile type of Hallervorden-Spatz's disease; ghost cells of Ule and Chou Shi-Ming [47]; filamentous profils of experimental neuroaxonal lesion by Chou et al. [3], are said that they show the figure simulate to vitamin E defficiency. Aleu' stated that the filamentous body of Gaucher's disease is common with that of Tay-Sach's disease.

Compared with fibrillar or filamentous structures found in the central nervous system, as already mentioned above, our description about the lamellar stru-

cture of Lafora body may be thought that fairly unique in its nature and character.

Further observation will be added in the near future about the relation of chemical composition and the structure, the metabolism in the process of lamella formation and participating enzymes.

SUMMARY

Brain biopsy from 15-year-old girl patient suffering from myoclonus epilepsy (Lafora type) was performed.

Biopsied specimen was studied by the light microscope, the phase contrast microscope and the electron microscope.

Light microscopic finding revealed many Lafora bodies and was completely consistent with the autopsy finding of siblings, already died of myoclonus epilepsy of Lafora type.

Phase contrast microscopic study disclosed the body consisted of two different layers, i. e. the external and the internal ones.

Electron micrograph of Lafora body revealed that its basic structure was consisted of lamella which composed of double electron dense lines measured about 5o to 100 Å thick and electron lucent interspace of similar thickness. Glycogen-like granules of about 250 to 500 Å in diameter were scattered throughout the body. Beside the basic lamellar structure, ribosomes, vacuolated rough surfaced endoplasmic reticulum and Golgi apparatus were also found in the large sized body.

Discussions were performed concerning following items.

1) Cortex without Lafora body.
2) Lafora body in the neuronal perikarya.
3) Small sized Lafora body.
4) Structures simulate to Lafora body.

The relation of abnormal vesicles and the process of Lafora body formation was especially considered.

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