Unusual Axons Found in Experimental Brain Edema of Cats*

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In this study on the fine structure of the experimental brain edema, many unusual axons were observed in both the grey and white matter of the cat’s edematous brain.

Although many who had investigated brain edema reported fine structural alterations of the edematous brain tissue, none of them noted such unusual axons as shall be described below.

In the present study an attempt was made to clarify when unusual axons begin to appear and how they transform and disappear through the process of edema.

The report will describe the fine structure of the unusual axons which appear at various stages of the experimental brain edema and discuss the morphological significance of these unusual axons.

Material and Methods

Fifty-five healthy adult cats weighing 2.0 to 4.5kg were used for this study.

Forty-one animals underwent the following operative procedures in order to produce the brain edema: According to the supratentorial epidural compression method of Ishii, Hayner, Kelly and Evans (1959), a balloon was inserted into the left frontal epidural space under intraperitoneal Nembutal anesthesia. A catheter was connected with the balloon and led to the outside through the burr hole and scalp. On the following day the balloon was very slowly inflated by injecting 1.4 to 2.3 ml of physiological saline solution through the catheter. The maximum inflation tolerable by the cat was then indicated by the onsets of right hemiparesis, anisocoria and the inactive body movement induced.

Fifteen of these experimental animals were epidurally compressed by leaving the inflated balloon in situ for various periods of time, from 2 to 48 hours, and then the material for electron microscopy was taken from both the grey and white matter under the Nembutal anesthesia.

After twenty-six of the animals were epidurally compressed for 48 hours, the balloon was deflated or removed. These cats were then kept alive for from six hours to six months, from which brain tissues were taken by the same method as stated above.

Tissue blocks were consistently removed from the left coronal gyrus and its adjac-
cent areas corresponding to the “somatic I” area of Woolsey (1947) over which of experimental animals the balloon had been inserted.

The fourteen animals which served as controls were anesthetized by the intraperitoneal administration of Nembutal and the material for electron microscopy was taken from the same sites as from the epidurally compressed animals.

After removal from the animals the material was immediately cut into small blocks with a razor blade in a drop of ice-cold fixative of Millonig (1961). After being fixed in the same solution for two hours these specimens were dehydrated in a series of graded ethanol and embedded in Epon epoxy resin (Luft 1961).

Thin sections cut on the Porter-Blum ultramicrotome were stained with lead hydroxide (Millonig 1961) and examined under the Hitachi HS-7S or HS-7 electron microscope.

After removing the material for electron microscopy, the entire brain was removed and fixed in 10% formalin for light microscopy. Frozen or paraffin-embedded sections were obtained from the coronal plane a little caudal to the region from where the material for electron microscopy was taken. Frozen sections were stained with Sudan III or with silver-impregnation technique (Nauta 1957) while paraffin-embedded sections were stained with hematoxylin-eosin, Klüver-Barrera or Bodian stains.

**Observations**

1. **Light microscopy**

In the specimens taken from animals with 48 hour compression hematoxylin-eosin, Klüver-Barrera and Bodian preparations revealed a sclerotic alteration of numerous neurons and sometimes an incomplete softening lesion in contact with the left lateral ventricle as is shown in the schematic illustration (Fig. 1). In Sudan III preparations a few fat granule cells were found in the softening lesion and the adventitial cells, especially of the cortex in the left hemisphere, were frequently fat-laden.

In the animals sacrificed a month after the decompression fat granules sometimes appeared to be phagocytosed in the adventitial cells only throughout both hemispheres but not in the microglia in the parenchyma. In Nauta preparations from cats sacrificed two months after the decompression a number of nerve fibers exhibited a drop-like disintegration in appearance and those fibers were distributed diffusely throughout the section, especially from the formerly compressed hemisphere. The preferential affection of the subcortical white matter underlying the balloon, however, was not observed. In animals which survived for three months after the removal of the balloon, neuronal sclerosis was detected in hematoxylin-eosin and Bodian prepara-
tions more extensively than in those with 24 hour compression. In the animals examined six months after the decompression, however, the neuronal sclerosis was scarcely observed.

2. *Electron microscopy*

In the specimens from the animals a variety of unusual axons, the appearance of which seemed to be, to some extent, related to the developing stages of brain edema, were frequently observed distributed in the sections. From their structural features, these unusual axons may be, as a matter of convenience, grouped into several types.

Type I (Fig. 2): This type of axon contained somewhat increased mitochondria, a few dense bodies and an unusual amount of neurofilaments in it. Although this type of axon began to appear during the compression for the first two to four hours, mitochondria and dense bodies in the axoplasm tended to increase more in number with the elapse of time during the compression.

![Fig. 2. An electron micrograph showing an unusual axon containing slightly concentrated mitochondria and dense bodies in the axoplasm. This pattern of axon was described as type I in the present observation. Cat brain with a 4-hour compression. × 13,000](image)

Type II (Fig. 3, 4, 5): In the case of the six hour compression, the axoplasmic structures, such as mitochondria, dense bodies, vesicles, neurofilaments and fine granules varying from 200 to 300 Å in diameter, were observed more concentrated than in type I. Axons of this type were more frequently encountered throughout the section than those of type I. Some of the axons, however, contained relatively increased neurofilaments which were arranged in various directions in the central
column of the unusual axon, as if those neurofilaments had pushed away the other axoplasmic structures such as described above toward the periphery. Some others, however, did not contain such abundant neurofilaments.

This type of axon was observed most frequently 24 and sometimes 72 hours after removing the compression for the period of two days. The maximum accumulation of the axoplasmic structures described here was found 24 hours after the decompression.

Type III (Fig. 4, 6, 7, 10): The axoplasm was filled with considerably dense and amorphous material or membranous material which resembled myelin in ap-
Unusual Axons in Experimental Brain Edema

Moreover, several axoplasmic structures similar to mitochondria, vesicles and dense bodies were sporadically distributed among the dense and amorphous axoplasmic material in some axons.

This finding was encountered in both non-myelinated and myelinated axons and the myelin sheath did not in general show the usual smooth lamellar structures, but revealed complex folding or irregular lamellar deterioration.

Fig. 4. In this electron micrograph several unusual axons are observed. An unusual myelinated axon at the upper left corner contains dense bodies and membranous material (designated as type III in the present work). At the lower left corner, a few unusual axons are seen including a number of axoplasmic structures (described as type II). Somewhat watery astrocytic processes at the right half of this micrograph show increased number of specific fine granules in their cytoplasm (A). A Ranvier's node distended is indicated by an arrow. Cat brain epidurally compressed for 48 hours. × 12,000
This type of axons was rarely found even in the animals with 48 hour compression but was found in high incidence in those which survived for from six hours to a month after the decompression.

Type IV (Fig. 8, 9): In this type of axons the region in which the axoplasm is usually located was sometimes occupied with a little amount of dense and amorphous material as in type III, though some other times it was seen to be empty. Myelin sheath of these unusual axons showed irregular lamellar exfoliation or destruction. This type of axons was rarely observed six hours after the decompression though it
was frequent after seven or fourteen days.

Type V (not illustrated): Six months after the decompression myelin sheath was more intensely exfoliated or destructed than that in type IV, or osmiophilic material similar to myelin was often observed distributed among the usual axons.

Type VI (Fig. 11) and VII (not illustrated): The former was seen to have a number of vesicles and vacuoles only. The latter contained multivesicular bodies and myelin-figured material in the axoplasm. These two types of axons rarely appeared six hours after the decompression.

Type VIII (Fig. 12): Six hours to three months after the decompression in several axons the myelin sheath showed splitting or exfoliative deterioration of the lamellar structure while the axoplasm exhibited the usual morphological patterns.

In addition to the eight types of axons as described above, another type of axon containing only a few neurofilaments and a number of fine granules was also found though very rarely. In this type the size of fine granules was as large as those in the cytoplasm of astrocyte and varied from 300 to 500 Å.

The dense bodies generally had no limiting membrane and were not larger than usual mitochondria in the central nervous tissue. Some of them showed similarity in fine structure to the usual mitochondria though these bodies were more dense in electron opacity than usual mitochondria.

Consistently throughout the present experiments, oligodendroglial and microglial cells were frequently observed showing their usual morphological patterns,
although it was sometimes difficult to distinguish one from the other. On the other hand, the astrocytes were morphologically unusual and, six months after the decompression, their cytoplasm contained many gliofilaments, specific fine granules or glycogen-like granules, well-developed endoplasmic reticulum and sometimes osmophilic material.

In animals served as controls axons with somewhat accumulated mitochondria and dense bodies were sometimes encountered. The accumulation of these structures was dispersed both in Ranvier's nodes and in the axon terminals, but those axoplasmic structures in controls were not so concentrated as those in the experimental group.

Discussion

Many reports published hitherto describe the fine structure of the spinal ganglion cells, ventral horn cells of the spinal cord or peripheral nerve after experimental nerve crushing, neurotomy or rhizotomy. Most of these reports (Andres 1961 and 1963, Blümcke, Niedorf and Rode 1966, Estable, Acosta-Ferreira and Sotelo 1957, Hartmann 1948 and 1954, Pannese 1963, Schilote 1964, Smith 1961, Tani 1964 and 1965, Vial 1958, Webster 1962, Wechsler and Hager 1962 and Wettstein and Sotelo 1963) demonstrate the unusual axons, in which several axoplasmic constituents such as mitochondria, vesicles and dense bodies are accumulated. Some of these authors interpreted that the accumulated axoplasmic compo-

Fig. 7. Axoplasm of this unusual axon is filled with dense membranous material. Note the enlarged intercellular space (ICS) and the irregular deterioration of myelin. This pattern of axon was described as type III. Six hours after the decompression. × 28,000
Unusual axons in Experimental Brain Edema

Components, especially accumulated mitochondria, represented the increase of energy requirement for regenerating the nerve fibers or for developing the growing cone. On the other hand, some others who studied Wallerian degeneration (Barton 1962, Honjin, Nakamura and Imura 1959, Nathaniel and Peace 1963, Ohmi 1961 and Thomas and Sheldon 1964) did not report such unusual axoplasmic components as mentioned above. Probably because of some difficulties encountered in the electron microscopic techniques in those days. The axons similar to the unusual ones in the present study were observed in the cuneate and gracile nuclei (Lampert, Blumberg

Fig. 8. Unusual axons with dense amorphous material are dispersed (arrows) and their myelin sheaths show an irregular deterioration. Some of them form an empty space (S) limited by myelin (type IV). At places, astrocytic processes containing a number of specific fine granules are observed (A). Seven days after the decompression. × 5,900

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Fig. 9. An electron micrograph revealing the lamellar exfoliative alteration of myelin. A little amount of dense amorphous material is detectable at the central portion. This was described as type IV. Two weeks after the decompression. × 6,500

Fig. 10. An electron micrograph showing an unusual axon, which contains dense amorphous and membranous material in the axoplasm and is included in the clear cell. This was described as type III in the present paper. A month after the decompression. × 14,000
Fig. 11. A large unusual axon containing many vesicles and vacuoles in the axoplasm is shown. This was described as type VI in the present work. *ICS* intercellular space. *G* specific fine granules in astrocytic processes. Six hours after the decompression. × 17,000

Fig. 12. A micrograph showing the splitting or exfoliative deterioration of myelin. This change was designated as type VIII. The axoplasm reveals the usual morphological pattern. Six hours after the decompression. × 25,000
and Pentchev 1964 and Lampert and Pentchev 1964) and the cerebral cortex
(Miyagishi 1966) of vitamin-E deficient rats and in the peripheral nerve and spinal
cord of rabbits suffering from allergic encephalomyelitis (Luse and McDougal 1960),
Ikuta (1966) observed such unusual axon in the perifocal brain tissue of a monkey
with an experimental brain tumor, and Hirata (1966) in the experimental ischemic
spinal cord of cats. The similar axons were also observed in the cerebral white mat-
ter of triethyltin-intoxicated rats (Hayano 1966), in the trigeminal ganglia of rats
-treated with Plasmocide (d’Agostino 1964), in the trigeminal ganglia of patients
-with trigeminal neuralgia (Kerr and Miller 1966). Furthermore the unusual axons
-were demonstrated in the lumbar sympathetic ganglion of an X-ray irradiated frog
(Pick 1965), in the connective served between ganglia of the insect abdominal cord
(Melamed and Trujillo-Cenoz 1963) and in the experimental puncture wound of
rabbit brains (Luse et al. 1960). Also in the corpus callosum of rats treated with
cyanide (Hirano, Levine and Zimmerman 1967) and in the white matter of rat on
whose surface a cake of dry ice was applied or on which a ouabain solution was
immersed (Okada and Tanaka 1966), these unusual axons were known to appear.

Therefore it is suggested that the unusual axons do occur also in the nervous
tissue without any relation to operative procedures such as crushing, neurotomy
etc., as is demonstrated in the present study in which the balloon is used.

Lampert, Blumberg and Pentchev (1964) and Lampert and Pentchev (1964)
found out multivesicular body and tubular structures in vitamin-E deficient rats and
Kerr et al. (1966) reported the proliferation of myelin in the trigeminal ganglion of
a patient with trigeminal neuralgia. These specific structures, however, were not
observed in the present study.

Fig. 13. Note some axoplasmic structures similar to mitochondria (arrows). They are more electron
dense than usual mitochondria. Six hours after the decompression. $\times 27,000$

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a patient with trigeminal neuralgia. These specific structures, however, were not
observed in the present study.
As also shown in the present study, the unusual axons at the late stage of edema exhibited the complex folding and destruction of myelin on which many had reported (BARTON 1962, BUNGE, BUNGE and Ris 1960, and HONJIN et al. 1959). The appearance of Schwann cells or phagocytes which contained fragmented myelin or osmiophilic material in the peripheral nervous tissue (ANDRES 1963, d'AGOSTINO 1964 and TANI 1964) was demonstrated, while in the central nervous tissue, specimens at the late stage frequently exhibited the occurrence of macrophages or reactive astrocytes which contained myelin debris, increased specific fine granules, gliofilaments (BUNGE et al. 1960, SHIRAISHI 1966 and TANI 1964), well-developed endoplasmic reticulum and ribosomes.

It is, therefore, assumed that the axons of type I and II may transform to type IV and V by way of type III and that the destructed myelin may be phagocytosed by macrophages or reactive astrocytes.

It was not demonstrated submicroscopically, however, from what type of axon type VI and VII were derived. Type VIII may transform to type V. In order to clarify the origin and the transformation of these types of axons, further study should be required.

In macroscopical or fluorescent microscopical study some reported the increased permeation of trypan blue (OKADA 1965), sodium fluorescein (HATANAKA 1963) and fluorescein labelled serum protein (KLATZO, MIQUEL and OTENASEK 1962) from the blood vessels to the brain tissue at the experimentally affected areas of the brain, which, they explained, could be attributed to the destruction of the blood-brain barrier.

In the present study, sclerosis or the shrinkage of neurons was observed expanded in light microscopic sections and fat granules frequently appeared in the adventitial cells in Sudan III preparations. Considering that LAMPERT et al. (1964) called the unusual axon which characteristically contained abundant mitochondria and dense bodies in the axoplasm as "dystrophic axon", it is apparently conceivable that these light microscopic findings may indicate the metabolic disturbance of the neuron and the abnormality of lipid metabolism in the experimental brain tissues.

In Nauta preparations a number of nerve fibers revealed a drop-like disintegration. Thus the unusual axons seem to be closely related to the degenerating or degenerated nerve fibers which may be the result of the metabolic abnormality of neurons and glial cells.

The past histochemical examinations of the pathological processes of nerve fibers revealed the increased activity of acid phosphatase at the proximal and distal stumps of the sectioned peripheral nerve as was reported by Barron and TUNCDAY (1962), HEINZEN (1949) and SAMORAJSKI (1957). And as to the different oxidative enzymes such as DPN- and TPN diaphorases, succinic dehydrogenase, cytochrome oxidase and ATPase, their heightened activities in the spinal cord and peripheral nerve were described at the early stage (FRIEDE 1959, KREUTZBERG 1963, KREUTZBERG and WECHSLER 1963 and KUMAMOTO 1965) and at the later stage (YOUNG 1966) after the experimental transection.

As submicroscopically demonstrated in the present study, judging from the appearance of the unusual axons and the macrophages or reactive astrocytes, it is reasonable to assume that their appearance may cause the heightened activities of
the different oxidative and lytic enzymes in the affected axons and their embracing macrophages. In the histochemical study on the local brain injury of rats, however, Mossakowski (1963) observed that the astroglia reacted with a remarkable increase of the succinic dehydrogenase activity after the initial decrease or disappearance of the activity, and Bachelard and Silva (1966) recently attempted to determine the activity of Na, K-activated Mg dependent ATPase in the fraction of the homogenate from the proximal and distal stumps of a cat's sciatic nerve after the experimental neurotomy, and observed that the decrease or marked loss of the activity appeared two to eight days after the neurotomy and that the activity showed a remarkable increase 16 to 32 days later. They described that the decrease or marked loss of activity might be masked by the proliferation of macrophage that had a heightened activity. As to the enzymatic activity some discrepancy is recognized among the reports mentioned above. Therefore the nature and activity of ATPase and other enzymes should be clarified in detail by a further study.

In the present study the increase of mitochondria in number was appreciated on their structural profiles, while Hartmann (1948) observed it by counting them according to the method of Rasmussen (Hartmann 1948) in the stained preparation for mitochondria taken from the ventral horn after the sciatic nerve section.

It is well known that after the constriction of nerves, the enzymatic damming (Friede 1959, Weiss and Hiscoe 1948) and the accumulation of mitochondria (Wettstein et al. 1963) are induced at the proximal stump. However, since the unusual axons are demonstrated also at the distal stump after the neurotomy (Blümcke et al. 1966 and Vial 1958), mitochondria are considered to be formed at the local region rather than to be moved down from the pericaryon by the axoplasmic stream (Blümcke et al. 1966). Hartmann (1954) and Causey and Hoffman (1955) describe that mitochondria are derived from the nuclear membrane. Napolitano and Fawcett (1958) report that they are from the pre-existing mitochondria. Geren and Schmitt (1954) assert that they are from axonal membrane, while Pannese (1966) observes the membranous whorls in close relation to mitochondria in the neuroblast of chick embryo. In the present study, however, no morphological evidence was obtained in regard to the development of mitochondria.

On the other hand, as was indicated by Webster (1962), the dense bodies were observed showing an internal structure similar to mitochondria. Thus it may be possible that dense bodies are derived at least in part from mitochondria.

In many ultrastructural aspects, the unusual axons in brain edema demonstrated here exhibit the similarity to those observed in the Wallerian degeneration reported in the past. Since, in the present study, such unusual process was induced not by the experimental lobotomy, corticotomy nor by other brain injuries, but by the brain compression only, it seems reasonable to consider that the mitochondrial accumulation does not imply the growing cone of axon or nerve regeneration. Judging from the observations such as the increase of glycogen-like granules or specific fine granules (Okada 1965) and of PAS-positive granules (Miquel, Klatzo, Meuzel and Haymaker 1963) in astrocyte, the swelling of astrocyte (Okada 1965 and Schröder and W echsler 1965), the accumulation of sodium and water contents (Pappius and Gulati 1963, Perret and Selbach 1940 and Stewart-Wallace 1939), the enlarged extracellular space (Ben-Schmuel 1964) of the white matter and the
increased anaerobic glycolysis (Herschkowitz and MacGillivray 1965) in the experimental brain edema, it is further conceivable that the metabolic alteration of glial cells induced by the brain hypoxia or cerebral circulatory disturbances following the compression may give rise to the occurrence of the unusual axon.

It is well known that mitochondria play an important role in maintaining the cell function as an active site of oxidative phosphorylation (Fernández-Morán, Oda, Blair and Green 1964 and Stasny and Crane 1964); if the unusual axons do actually represent the degenerating or degenerated fibers, it is difficult to understand the relationship between degeneration of axon and the accumulation of mitochondria. However, it is assumed that the axon or neuron may react in various manners to the intensity and the duration of some stimulus in order to balance the disturbed cell activity and that, therefore, the unusual axon at the early stage does possibly return to the normal appearance under the improved circumstances.

**Summary**

In a study of the experimental brain edema using the balloon-compression method, unusual axons characterized by numerous mitochondria, vesicles, dense bodies, amorphous material and neurofilaments were demonstrated by electron microscopy. According to their structural features which associated with the stages of the brain edema, the unusual axons were classified into eight types. It was suggested that type I and II might transform to type IV and V by way of type III. The unusual axons accorded in many structural respects with those of nerve degeneration reported in the past. Therefore it was suggested that the unusual axons were closely related to the nerve degeneration, which was supported by the results of the light microscopic observation in the present study.

The appearance of the unusual axons may be attributed to the metabolic alterations of glial cells induced by the brain hypoxia or cerebral circulatory disturbance following the compression by which the nourishment of nerve cell may be disturbed.

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破壊が著しく、細胞がよく似た電子密度の高い物質が通常の線維の間に観察されたりした。結局、これらの特異な形態を示す線維は一連の変化過程にあるもので、これまでに脳浮腫の研究で報告されているように、星状膠細胞の膨化やその細胞質中におけるグリコゲン類特殊顆粒の増加、毛細血管透過性亢進、白質の細胞間隙の拡大などから、圧迫のために生じた局所的循環障害による星状細胞の代謝異常、次いで神経細胞にも代謝異常が起こり、まず線紡学的にミトコンドリアの増加をもって反応した神経細胞は、この異常に対抗しきれず、特異な形態を示すこれらの線維は変性過程を歩むに至ったものと考えられる。このことは二次変性を主としたこれまでの多くの研究や、本研究の光顕的所見からも支持される。

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