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Ultrastructural Observations on Axonal Swelling in the Human Gracile Nucleus

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Summary. The morphology of the spheroids in human gracile nuclei was studied by light and electron microscopy. Various spheroids encountered in the present study could be classified into three types based on the internal structure: The first one was chiefly composed of many irregular homogeneous dense bodies, multivesicular bodies and mitochondria. The dense bodies seemed to deposit multicentrically in an axon in the early stage of "balloon" formation and coalesce to form larger ones. The second was characterized by a marked accumulation of closely approximated mitochondria and dense concentric bodies. In the third the most characteristic findings were neurofibrillary accumulation and aggregations of dense bodies. These findings showed some divergence from those of dystrophic axons and the last two mimic those of degenerative or regenerative axons, which suggested that axonal swelling (including dystrophic axon) is not characteristic reaction of specific disease but rather nonspecific one to a variety of noxious stimuli.

With light microscope, it was difficult to distinguish balloons with different structures since they were quite diverse and manifold in their shape, size, appearance and stainability. Intra-axonal corpora amylacea were seen in most cases and their incidence appeared to be nonspecific for any diseases.

Key words: Axonal swelling – Spheroid – Dystrophic axon – Ultrastructure – Human gracile nucleus.

Introduction

The phenomenon of axonal swelling has attracted attention because it occurs in a wide variety of conditions including trauma, neoplastic infiltration, vascular disturbance, degenerative or demyelinating disorders and metabolic disease. It is uncertain whether the lesions is regenerative or degenerative. In a previous study (Yagishita, 1978) we found that the morphology of axonal "balloons" in human disease shows some differences from those found in experimental

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Table 1

Case	Age	Sex	Clinico pathological diagnosis
77–02	72	M	Schizophrenia
77–03	63	M	Stomach cancer
77–04	72	F	Acute heart failure
77–06	30	F	Spinal cord injury and Spina bifida
77–10	47	F	Glioblastoma multiforme
77–11	63	F	Retroperitoneal bleeding (traumatic)
78–00	49	M	Encephalomalacia
78–02	53	M	Sudanophilic leukodystrophy
77–54	74	M	Lung cancer
77–74	66	M	Acute pancreas necrosis
78–07	58	M	Progressive spinal muscular atrophy
78–08	74	M	Cerebral contusion

animals (Lampert, 1967). The histology and topography of axonal dystrophy have been extensively investigated by Fuzisawa (1967), Seitelberger (1971) and Jellinger (1973) but systematic ultrastructural studies on axonal swellings in the gracile nucleus has not been reported. The purpose of this study is to clarify the ultrastructural findings in axonal swellings in this nucleus.

Material and Methods

The gracile nuclei of autopsy cases were used for this study. The total number of cases examined was 12, and their age, sex and clinicopathological diagnosis are listed in Table 1.

For electron microscopic observation, the medulla oblongata of each case was coronally sectioned at the level of the obex and immediately immersed in 2.5% glutaraldehyde solution. The gracile nucleus was dissected and the tissues were fixed in glutaraldehyde and postfixed in 1% osmium tetroxide. After serial dehydration in graded alcohol, the tissue was embedded in epon mixture. Semi-thin sections were screened by light microscope. Thin sections were treated with uranyl acetate and lead citrate and were observed by an Hitach HU-12 electron microscope. More than ten different sections were observed in each case.

For light microscopy, all samples were embedded in paraffin wax. The routine stains employed were hematoxylin-eosin (HE), periodic acid Schiff (PAS), Masson's trichrome, luxol fast blue (LFB), cresylviolet and Bodian. The histochemical methods applied to selected cases will be described later.

Results

There were many axonal swellings and spheroids in most cases. Spheroids were diverse in their shape, size, appearance and stainability. In HE-preparations they appeared in varying shades, from deeply reddish to pale pink, and were finely granular. Sometimes a clear space was seen around the spheroids. Their shapes varied from round to oval; they were sometimes irregularly elongated or grotesque in shape. Their size ranged from several micra to over 100 micra in diameter. Most spheroids gave strongly positive results with PAS-prepara-

Table 2. Histochemical properties of spheroids

H.E.	eosinophilic
PAS	+ve to −ve
PAS-saliva	+ve to −ve
Masson	red or blue
LFB	+ve to −ve
Ziehl-Nielsen	+ve to −ve
Toluidine blue	
PH 2.5	blue
PH 4.1	−ve
PH 7.0	−ve
Best carmin	+ve to −ve(granular)
Nile blue	blue
Congo red	+ve to −ve
Alcian blue	−ve
Berlin blue	−ve
Colloid iron	pink or blue
Crecylviolet	blue violet
Turnbull blue	−ve
Bodian	+ve
Sudan black B	grayish brown
Sudan 111	−ve

+ve = positive, −ve = negative

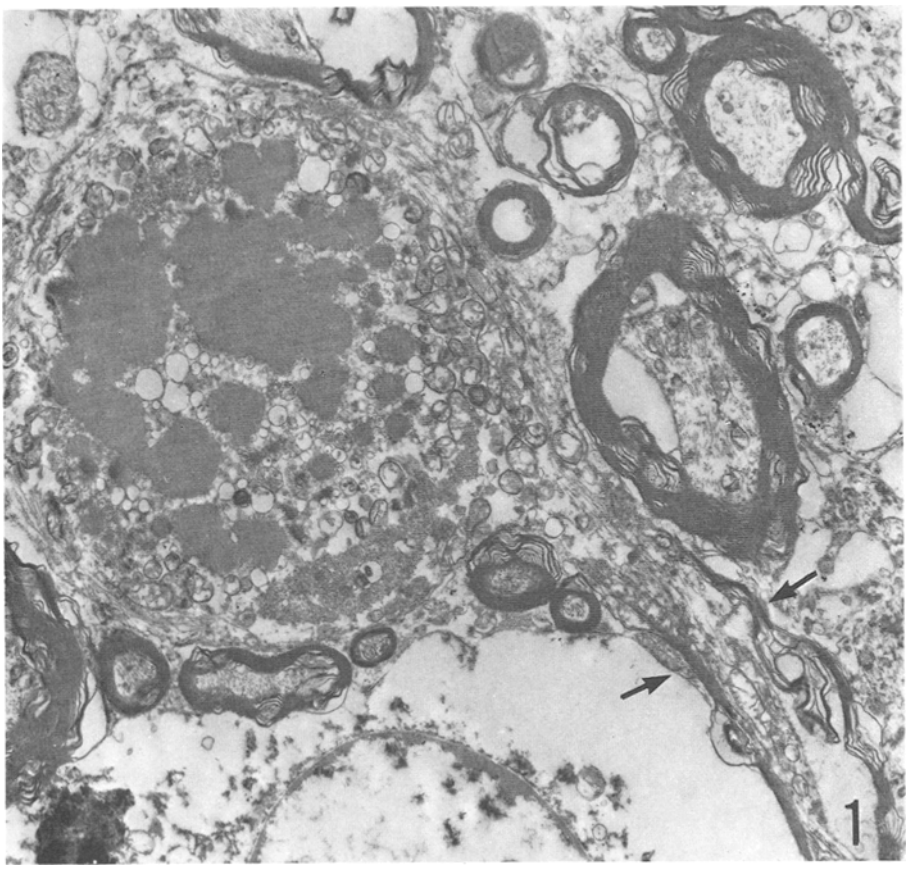

Fig. 1. A spheroid consisting of multiple homogeneous dense bodies, multivesicular bodies and mitochondria, It is connected to a myelinated axon (*arrow*). ×7,000

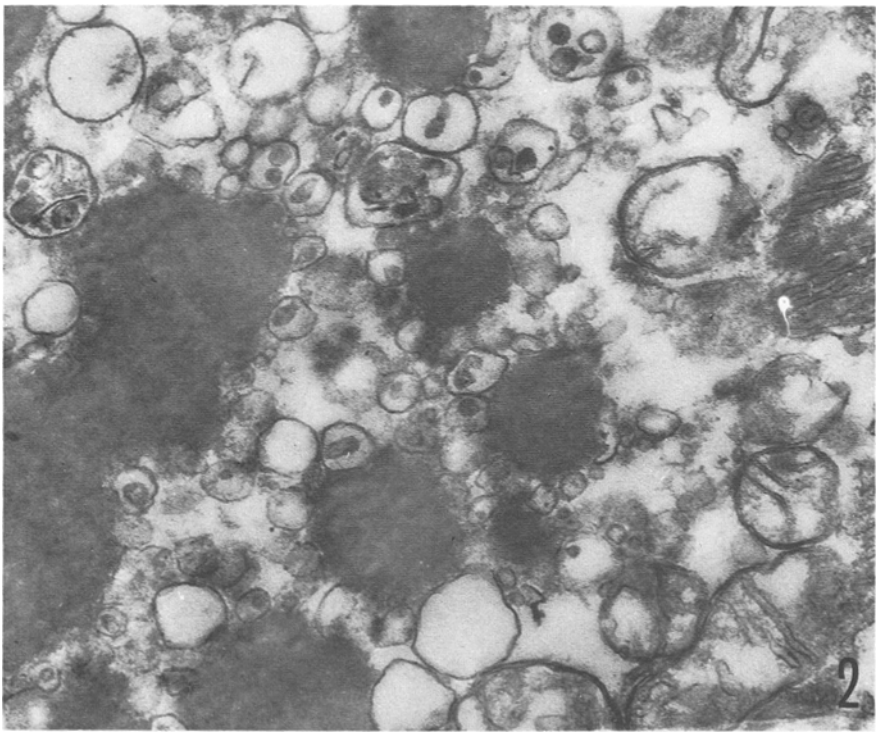

Fig. 2. Higher magnification of Fig. 1. HDBs are not membrane-bordered and not related with any organelles. ×33,000

tions, some were PAS-negative. All PAS-positive spheroids were partly digested by saliva. Some were also stained positive with LFB and Sudan black B. The histochemical properties are shown in Table 2. It is likely that spheroids contained polysaccharide, lipid and protein as its constituents.

Electron Microscopy

Many spheroids and axonal swellings were seen. The spheroids could be tentatively classified into three types based on their internal structure; the first consisted of many homogeneous dense bodies, multivesicular bodies and mitochondria (HDB). HDBs seemed to develop multicentrically in an axon (Figs. 1 and 2) and coalesce to form larger bodies. Occasionally a single large HDB occupied the whole area of an axoplasm (Fig. 4). HDB seemed to be not membrane-bordered and appear among the cellular organelles. Sometimes "balloons" were invested by a myelin sheath or connected to a myelinated axon, indicating their localization within an axon (Fig. 1). The second type of spheroid was characterized by a marked accumulation of closely approximated mitochondria and dense bodies (Fig. 6). This type rarely appeared in a myelinated axon. Mitochondrial matrix was abnormally dense and the membranes were more

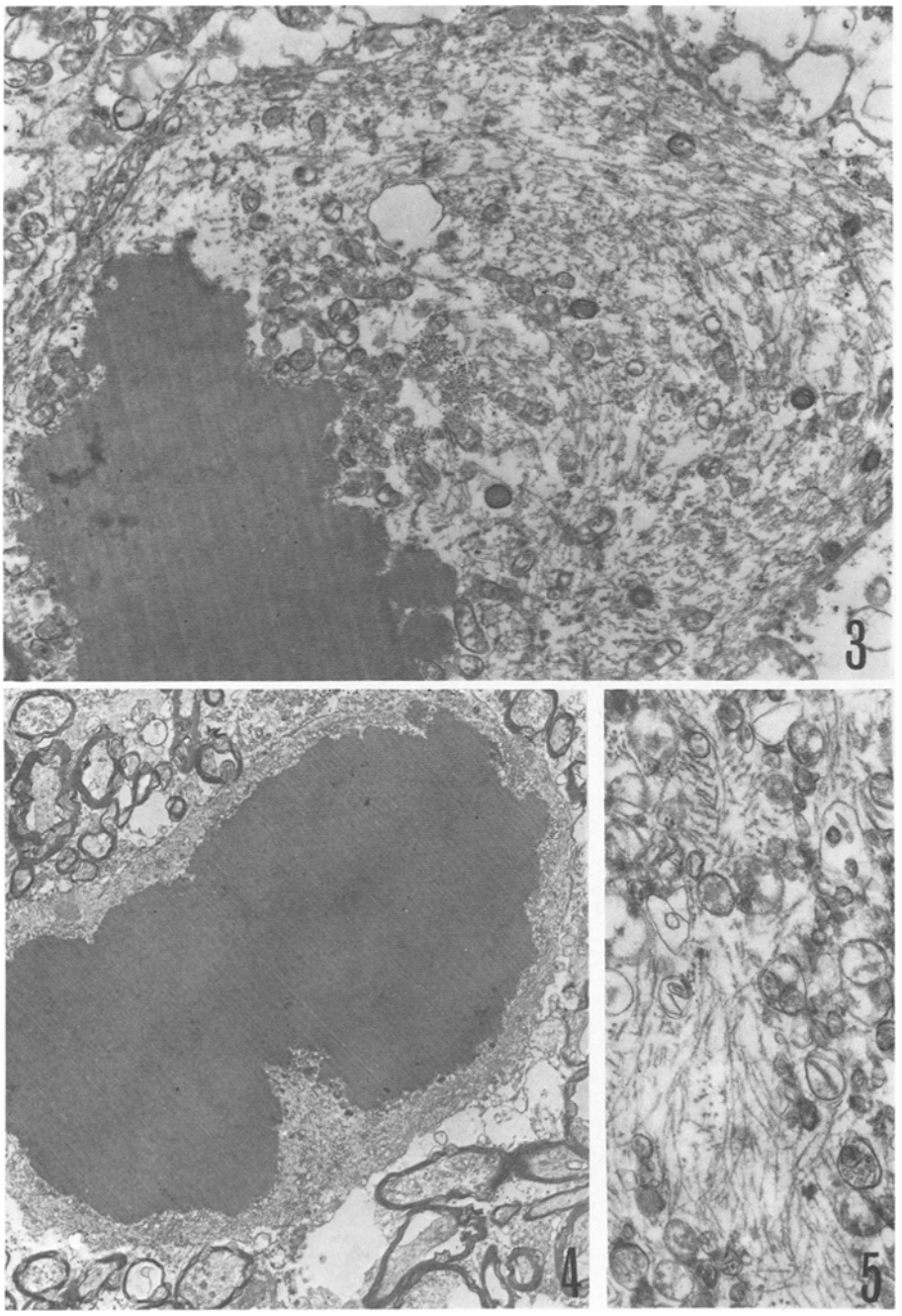

Fig. 3. Coexistence of filamentous body and large HDB. ×8,000

Fig. 4. A large HDB in the center of a spheroid. ×2,200

Fig. 5. A part of a filamentous spheroid. Many neurofilaments and degenerating mitochondria are seen. ×15,000

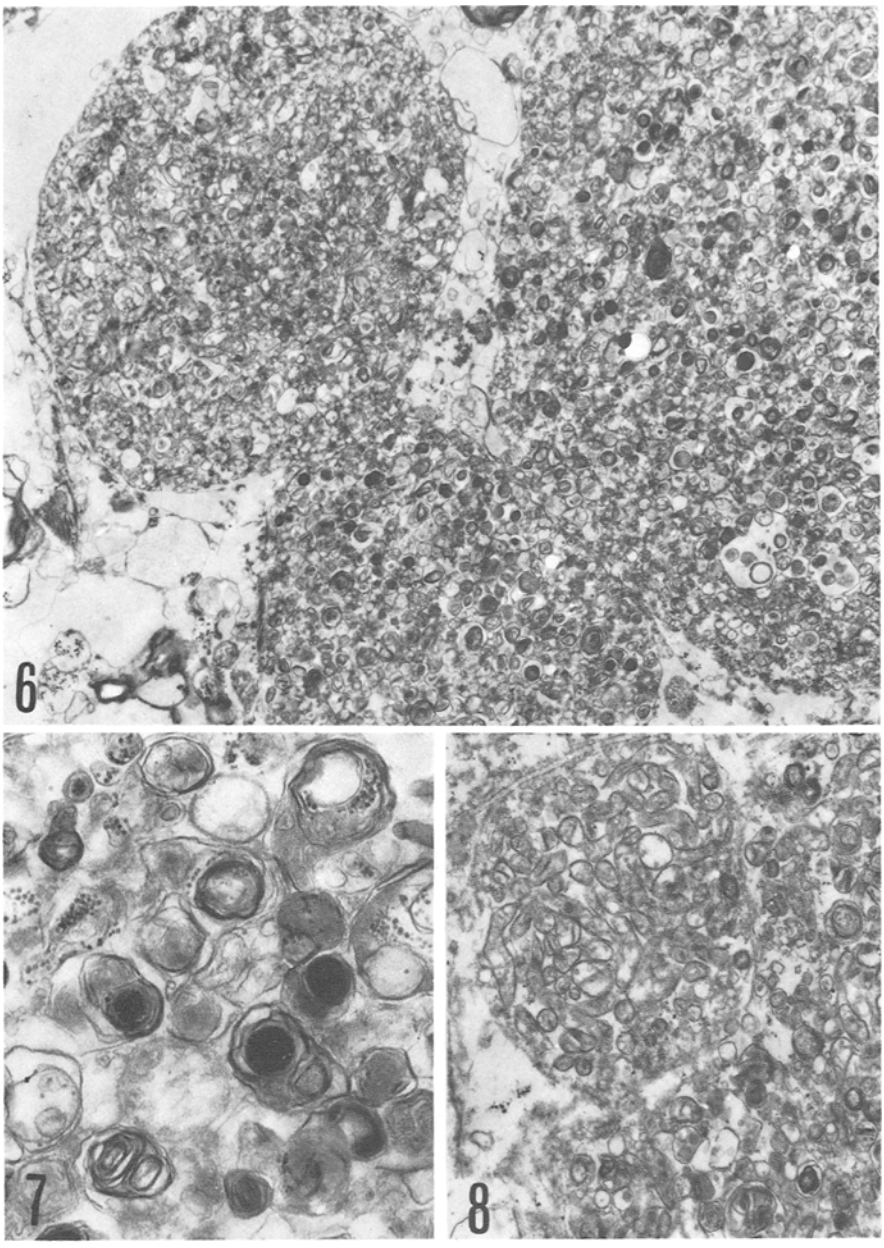

Fig. 6. Three balloons consisting of accumulations of concentric bodies and degenerating mitochondria. ×6,000

Fig. 7. Many dense bodies with concentric laminations. Varying numbers of glycogen granules are seen between lamellae or in the center. 28,000

Fig. 8. Mitochondrial aggregations in a balloon. ×8,000.

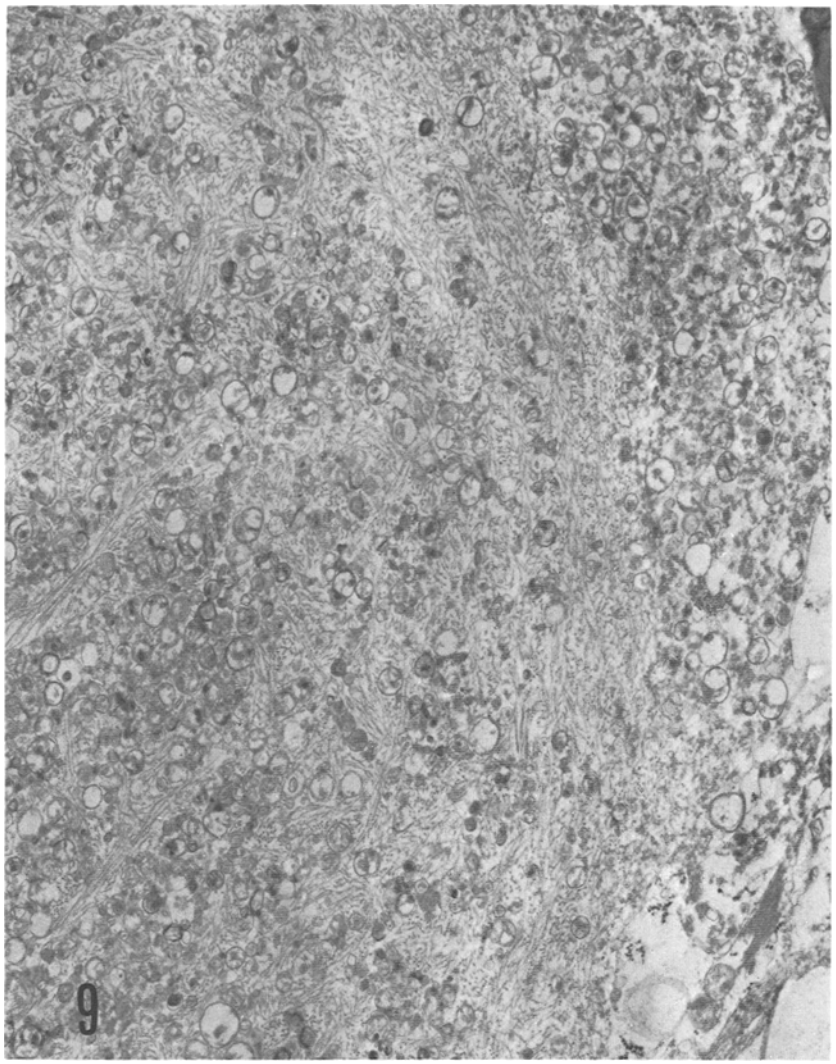

Fig. 9. Neurofibrillary accumulation and many dense bodies in a spheroid. The amount of fibrillary material varies in different portions in a body ×8,500

or less distorted (Fig. 8). The majority of the dense bodies were round or oval in shape and presented concentric laminations. Some had a dense central core. These dense bodies contained varying numbers of glycogen granule between their lamellae or in their center (Fig. 7). This type of spheroid (CB) was virtually devoid of other organelles. In the third type fibrillary accumulations and aggregations of dense bodies were the most prominent features (Fig. 9). The fibrils were composed of interlacing fine filaments resembling neurofilaments (Fig. 5). The amount of fibrils varied between "balloons" and even in different portions in the same balloon. They were usually over 50 μ in diameter and were generally

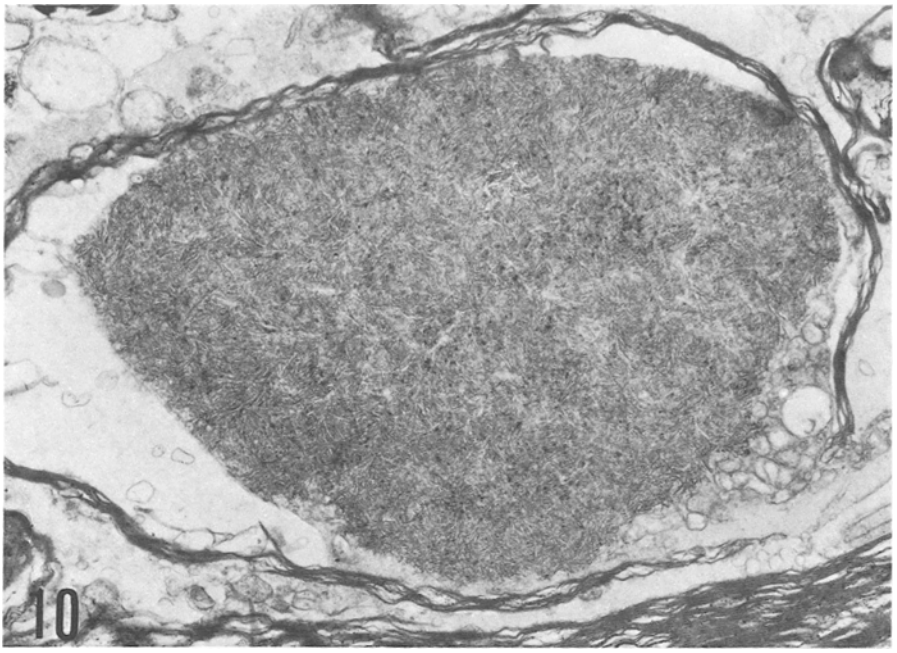

Fig. 10. Intra-axonal corpus amylaceum in a myelinated axon. ×7,000

Table 3. Occurrence of spheroids and Intra-axonal corpora amylacea

Case	Age	Sex	HDB	CB	F	IACA
77–02	72	M	+	−	−	+
77–03	63	M	−	+	−	−
77–04	72	F	+	+	+	+
77–06	30	F	+	−	−	−
77–10	47	F	+	+	−	−
77–11	63	F	+	+	+	−
78–00	49	M	+	+	−	−
78–02	53	M	+	+	+	+
77–54	74	M	−	−	−	−
77–74	66	M	+	+	−	+
78–07	58	M	−	−	−	+
78–08	74	M	−	−	−	−

enclosely by a single membrane but occasionally invested by thin myelin or remnants of a myelin sheath. CB-spheroids often clustered. Filamentous spheroids and HDB were isolated and scattered between large numbers of neuropils. Sometimes stacks of filaments and HDB coexisted in the same body (Fig. 3). Interconnected tubules, stacked membrano-tubular profiles, and the alternating layered membranes which are classical features of dystrophic axon in infantile neuroaxonal dystrophy, were rarely seen.

A few axons were studded with many myelin figures, aggregations of glycogen granules, accumulation of mitochondria and neurofilaments but this was not constant feature. Intraaxonal corpora amylacea were present in most cases. The occurrence of these types of spheroids and intra-axonal corpora amylacea is shown in Table 3. Each case was marked only as positive or negative since one cannot cover large quantities of the gracile nucleus by electron microscopic observation, and quantitative assessments are not possible.

Discussion

Many axons in the gracile nucleus examined in the present study were found to be enlarged, by three types of spheroids. HDB appeared to develop multicentrically in an axon and coalesce to form larger bodies, finally occupying the whole area of an axoplasm. They were not membrane-bordered and seemed to be unrelated to any organelles. Jellinger (1973) described a dense homogeneous body in the center of spheroid and considered that it was an old event in spheroid formation. This dense body is virtually identical to HDB in the present study. Thus it is likely that HDB may develop in the early stage of spheroid formation. Although it can generally be assumed that axonal swellings in the gracile nucleus belong dystrophic axons the occurrence of "filamentous" and "CB-spheroids" in this nucleus poses some questions. Both spheroids have been observed in symptomatic lesions such as neoplastic infiltration and cerebrovascular disease and should not be considered as important features of the dystrophic axon.

Balloons in the gracile nucleus of aged rats (Fuzisawa, 1978) seemed to have some morphological differences from those seen in human beings. HDBs are rarely seen in axonal swellings in infantile neuroaxonal dystrophy, although Jellinger (1973) described them in the axons. The ultrastructure of balloons in other areas is scarcely reported in human disease and those reports which exist show some differences from those seen in human gracile nucleus; they are either "filamentous" or CB-spheroids, virtually no HDBs are seen (Carpenter, 1968; Liu et al., 1974; Ule, 1972). Thus the morphology and pathological significance of balloons in other areas requires a further systemic investigation.

Another interesting finding was the presence of filamentous inclusions in a myelinated axon, the ultra-structure of which was identical to that of a corpus amylaceum. Intra-axonal corpora amylacea have been reported in various human diseases and also in normal individuals (Suzuki et al., 1971; Pettito et al., 1973; Anzil et al., 1974; Ulrich et al., 1977; Takahashi et al., 1975; Yagishita et al., 1977; Fukuhara et al., 1977). It is likely that this inclusion is not specific for any disease but that it is related to some peculiar aspect of chronic axonal degeneration, with some correlation with aging.

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