Acta Neuropathol (1988) 76:217–221

Acta Neuropathologica

Regular papers

Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses

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Summary. We systematically studied the enteric nervous system of the alimentary tract in seven patients with Parkinson's disease. In all patients, characteristic inclusions histologically and ultrastructurally identical to Lewy bodies were found in Auerbach's and Meissner's plexuses. They were most frequent in the Auerbach's plexus of the lower esophagus. Lewy bodies were found in 8 out of 24 age-matched nonparkinsonian patients. However, they were obviously small in number. These findings clearly indicate that the plexuses are also involved in Parkinson's disease.

Key words: Parkinson's disease – Lewy body – Auerbach's and Meissner's plexuses

Since James Parkinson's original description of paralysis agitans [19], a variety of autonomic symptoms have come to be well known as constant clinical features of Parkinson's disease: sialorrhea, seborrhea, postural hypotension and alimentary symptoms such as dysphagia, heartburn and constipation are common [1, 6]. Postmortem studies of patients with Parkinson's disease have shown pathological changes in the hypothalamus [13], dorsal vagal nucleus and other brain stem nuclei [5, 15, 18], intermediolateral nuclei of the spinal cord [17, 24], as well as in the peripheral sympathetic ganglia [3]. However, there have been only a few reports with respect to the morphological changes present in the enteric nervous system [12, 20, 25]. We, therefore, attempted to examine systematically the enteric nervous system of the alimentary tract in patients with Parkinson's disease, using Lewy body (LB) formation as a marker of neuronal degeneration.

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In this paper, we describe the occurrence of LBs in nerve cells and nerve cell processes in Auerbach's and Meissner's plexuses in patients with Parkinson's disease, and discuss the implications and possible significance of this finding.

Materials and methods

The seven autopsied patients studied were selected on the basis of their clinical histories and neuropathological findings. The diagnosis of Parkinson's disease was confirmed histologically by the presence of numerous LBs and neuronal degeneration in the substantia nigra, locus ceruleus and other brain stem nuclei [18]. The age of the patients ranged from 56 to 75 years, averaging 62.4 years. The duration of illness was from less than 1 year to 27 years. We also examined 24 age-matched nonparkinsonian patients from 50 to 81 years of age, averaging 66.4 years, in whom no LBs were found in the central nervous system (CNS).

Tissues were fixed in 10% formalin and embedded in paraffin. Serial 4-μm sections were cut from block samples of 11 different levels of the alimentary tract: upper, middle and lower esophagus, corpus of the stomach, duodenum, jejunum, ileum, and ascending, transverse and descending colon, and rectum. In one patient with Parkinson's disease the tissue preparation was limited to the middle esophagus, stomach, jejunum, ileum, and ascending colon. First, fourth, seventh and tenth sections were chosen from serial sections for each level and were stained with hematoxylin and eosin. Bodian's, periodic acid-Schiff (PAS), Congo red, Azan-Mallory, Masson's trichrome and Klüver-Barrera stainings were also performed when necessary.

For electron microscopy, selected light microscopic sections were recycled according to the method of Rossi [21]. The sections containing LBs were immersed in xylene until separation of the cover glass. The sections were rehydrated in a graded ethanol series and then washed with 0.1 M phosphate-buffered solution (PBS). Following postfixation with 1% osmium tetroxide in PBS, the sections were dehydrated in a graded ethanol series up to absolute ethanol. One drop of Epon 812 was placed on each section and then a previously polymerized Epon block was put over it. After polymerization at 60°C, the Epon block was removed from the glass slide. Ultrathin sections were cut from the area containing LBs, stained with uranyl acetate and lead citrate, and examined by electron microscopy.

Table 1. Occurrence of Lewy bodies (LBs) in the enteric nervous system of the alimentary tract in Parkinson's disease

Case no.	Age, sex (duration in years)	Esophagus			Sto.	Small intestine			Colon			Rec.
		Upper	Middle	Lower		duo.	jej.	ile.	asc.	trans.	des.	
1	56, M (18)			●● ●● ●●●			○				○○	
2	56, F (<1)			●	●							○
3	59, M (16)	●	●	●● ●● ●●●								
4	61, M (7)	N.E.		N.E.		N.E.			○	N.E.	N.E.	N.E.
5	65, M (8)		●●● ●●● ●●●	●●●● ●●●● ●●●●★ ●●●●★	●							
6	65, M (3)		●	●● ●●		● ●●						
7	75, F (27)			●●			● ●●		●			●●

●, One intraneuritic LB in Auerbach's plexus; ○, one intraneuritic LB in Meissner's plexus; ★, one intracytoplasmic LB in Auerbach's plexus
N.E., Not examined; Sto., stomach; duo., duodenum; jej., jejunum; ile., ileum; asc., ascending; trans., transverse; des., descending; Rec., rectum

Results

LBs were found in Auerbach's and Meissner's plexuses in all seven patients with Parkinson's disease (Table 1). By light microscopy, most of the LBs appeared as homogeneous or laminated, spherical or elongated inclusions with a clear surrounding halo (Figs. 1a, 2a). They ranged in diameter from 3 to 12 μm. The staining reactions were very similar to those previously reported in the CNS [11]. They were slightly argyrophilic in Bodian's preparations and stained deeply red with Azan-Mallory. They were usually intraneuritic and were more frequent in Auerbach's myenteric plexus than in Meissner's submucosal plexus. The ganglion cells often contained a varied amount of lipofuscin granules in the cytoplasm, and rarely showed vacuolated, swollen or chromatolytic cytoplasm. However, no significant loss of neurons was noted.

Ultrastructurally, the LBs revealed two different forms. The common form was entirely composed of filamentous material (Fig. 1b). The filaments were randomly arranged, and tended to aggregate in the central portion of the body. The other form consisted of an inner core with extremely electron-dense granular material and an outer zone with radially oriented filaments (Fig. 2b). In both forms, the constituent filaments were approximately 10–12 nm in diameter. There was no limiting membrane separating them from the surrounding cytoplasm. It was difficult to determine whether the nerve cell processes containing LBs were axons or dendrites.

Although LBs were distributed widely in the alimentary tract, they were most frequent and numerous in the esophagus, especially its lower portion. There was no significant correlation between the occurrence of LBs and the duration of the illness.

LBs were found in 8 out of 24 age-matched nonparkinsonian patients. They were very small in number and were found only in Auerbach's plexus (Table 2).

Discussion

In all seven patients with Parkinson's disease studied here, LBs were found in Auerbach's and Meissner's plexuses. They occurred predominantly in neurites, similar to the case of those in the peripheral sympathetic ganglia [3]. Histologically, they appeared very similar to those seen in the CNS except for their size. The diameter of LBs reported here ranged from 3 to 12 μm, and were thus apparently smaller than those in the CNS, which have been reported to range from 5 to 25 μm [11] or from 2 to 40 μm [2] in the substantia nigra. Ultrastructurally, the multilaminated type of LB was composed of an electron-dense core with surrounding radially arranged filaments. These structures are identical to those reported in the substantia nigra

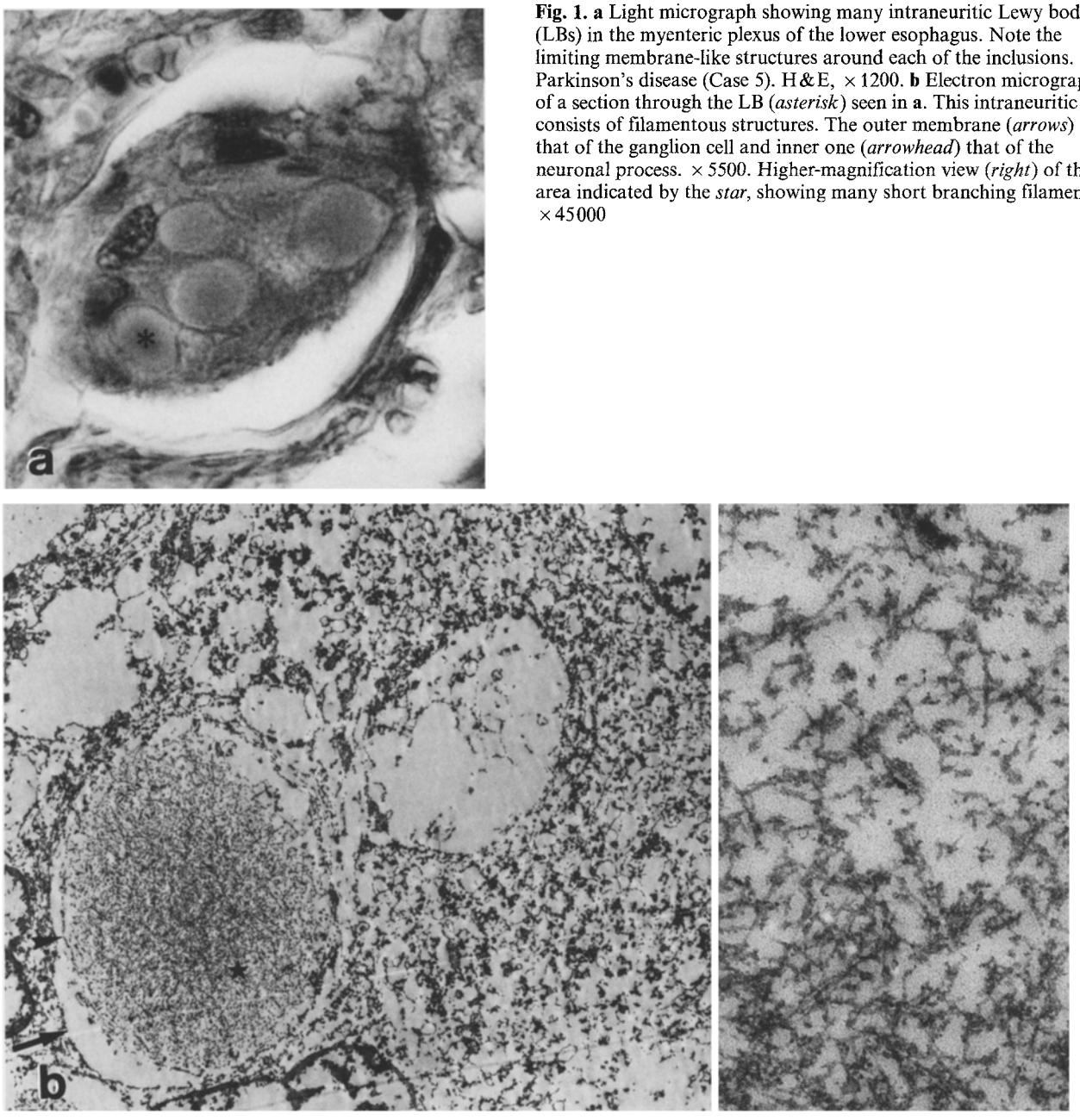

Fig. 1. a Light micrograph showing many intraneuritic Lewy bodies (LBs) in the myenteric plexus of the lower esophagus. Note the limiting membrane-like structures around each of the inclusions. Parkinson's disease (Case 5). H&E, ×1200. **b** Electron micrograph of a section through the LB (*asterisk*) seen in **a**. This intraneuritic LB consists of filamentous structures. The outer membrane (*arrows*) is that of the ganglion cell and inner one (*arrowhead*) that of the neuronal process. ×5500. Higher-magnification view (*right*) of the area indicated by the *star*, showing many short branching filaments. ×45000

[4]. On the other hand, the homogeneous type of LB was composed of randomly arranged filamentous material. This type of structure was quite similar to that of the "fibrillar" or "filamentous" LBs in the substantia nigra [22] and stellate ganglion [8]. In both forms, the constituent filaments, 10 to 12 nm in diameter, were the same as those of LBs in the CNS [4].

To our knowledge, the occurrence of LBs in Auerbach's plexus in Parkinson's disease was first reported by Qualman et al. in 1984 [20]. The latter authors described LBs in 2 out of 22 patients with Parkinson's disease and in 2 out of 8 patients with achalasia. Although the inclusions were similar to LBs histologically, their ultrastructural findings appear to be different from those of the LBs described here and those previously reported elsewhere [4, 8, 22]. More recently, Kupsky et al. [12] reported LBs in colonic ganglion cells in a patient with Parkinson's disease and megacolon.

The occurrence of autonomic symptoms of the alimentary tract is well known in Parkinson's disease [1, 6]. Diminished peristalsis and dilatation of the esophagus, megacolon, and dilatation of the small intestine have also been reported radiologically [10,

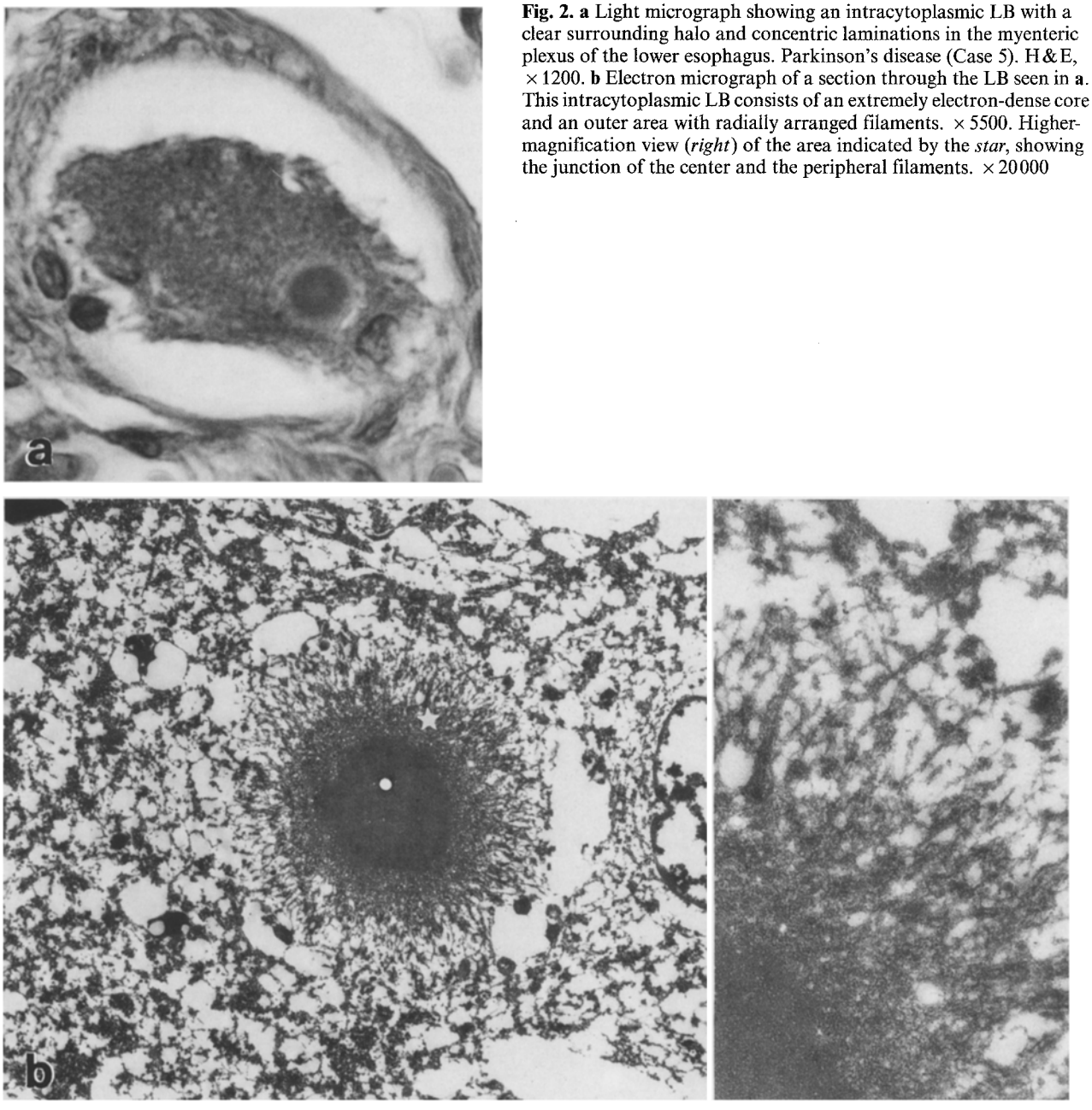

Fig. 2. a Light micrograph showing an intracytoplasmic LB with a clear surrounding halo and concentric laminations in the myenteric plexus of the lower esophagus. Parkinson's disease (Case 5). H&E, ×1200. **b** Electron micrograph of a section through the LB seen in **a**. This intracytoplasmic LB consists of an extremely electron-dense core and an outer area with radially arranged filaments. ×5500. Higher-magnification view (*right*) of the area indicated by the *star*, showing the junction of the center and the peripheral filaments. ×20000

14, 16]. In the present study, we found LBs to be widely distributed in the alimentary tract, especially in Auerbach's myenteric plexus of the lower esophagus. At present, however, we do not have enough data to discuss the relationship between the frequency of LBs and the presence or the degree of clinical symptoms. Dysphagia had been noticed in two of the patients with Parkinson's disease (cases 1 and 7) and one of them (case 1) had also shown severe constipation.

In the present study, LBs were also found in 8 out of 24 age-matched nonparkinsonian patients. In the CNS, LBs have been occasionally found in aged patients without recognized symptoms of Parkinson's disease [7, 23]. The significance of our finding appears to be the same as that in the CNS, suggesting that there exists a close relationship between the disease process of Parkinson's disease and aging.

The alimentary tract is innervated by Auerbach's myenteric and Meissner's submucosal plexuses, although it also receives nerve fibers from sympathetic and parasympathetic ganglia. The majority of the axons observed in Auerbach's and Meissner's plexuses have been considered to be of intrinsic origin [9]. Our present study clearly indicates that Auerbach's and

Table 2. Occurrence of Lewy bodies in the enteric nervous system of the alimentary tract in 8 out of 24 control cases

Case no.	Age, sex	Esophagus			Sto.	Small intestine			Colon			Rec.
		Upper	Middle	Lower		duo.	jej.	ile.	asc.	trans.	des.	
1	61, M						●					
2	62, F					●						
3	62, M							●				
4	66, M			●●								
5	69, M					●						
6	71, M					●●						
7	77, M			●								
8	81, F			●●								

Abbreviations as in Table 1

Meissner's plexuses are also involved in the disease process of Parkinson's disease.

Acknowledgements. We wish to express our appreciation to Mr. T. Ichikawa, Mr. K. Kobayashi, Mr. S. Egawa and Ms S. Sekimoto for their technical assistance and Ms K. Murayama and Mrs. Y. Tanahashi for their help in preparing the manuscript.

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Received December 29, 1987/
Revised, accepted February 29, 1988