PHASE AND ELECTRON MICROSCOPIC OBSERVATIONS OF LEWY BODIES AND MELANIN GRANULES IN THE SUBSTANTIA NIGRA AND LOCUS CAERULEUS IN PARKINSON'S DISEASE* †

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Lewy (1, 2) described spherical bodies in neurons of the substantia innominata and in the dorsal vagal nucleus in idiopathic paralysis agitans, which were later seen by Trétiakoff (3) in the pigmented cells of the midbrain. Trétiakoff (3) called the inclusions Lewy bodies and more specifically associated them with Parkinson's disease. This subject was reviewed by many investigators including Lewy (4), and more recently by Greenfield and Bosanquet (5), who added a number of new cases with similar inclusions and pointed out the staining qualities of these bodies. The less common occurrence of similar bodies in other conditions has been described by Lipkin (6) and Okazaki et al (7). Nevertheless, the frequency of their occurrence in Parkinson's disease has been made clear in the literature (5, 6) and again was substantiated in this Medical Center in brains examined in the Parkinson's Disease Information and Research Center Brain Bank. The high incidence of Lewy bodies in Parkinson’s disease warrants further elucidation of their structure.

It is generally accepted that there is frequently a decrease in melanin in the pigmented nuclei of the brain stem in Parkinson's disease, but it is not known whether this is the result of nerve cell breakdown alone, or whether there is a preceding change in the melanin. The fine structure of melanin granules has been studied in many sites, including skin (8), hair (9), retina (10), and normal substantia nigra (11). While recognizing the limitations of autopsy material for electron microscopy, it seems appropriate to employ this material for the study of Lewy bodies and melanin granules, since they remain relatively well preserved and because the neural tissues containing them are at present relatively inaccessible for biopsy. We studied these structures from the standpoint of correlating their morphology in light, phase, and electron microscopy.

MATERIALS AND METHODS

The brains of 2 patients who had Parkinson's disease and 3 normal brains, were obtained at autopsy and fixed in 10 per cent formalin or in 6.25 per cent glutaraldehyde in

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phosphate buffer. The external appearance of the brains was described, and the brains were sectioned in the coronal plane. Blocks of tissue were removed from all major lobes of the brain, the basal ganglia, brain stem, and cerebellum for paraffin and colloidion embedding. For light microscopic study the sections were stained with hematoxylin and eosin, Giemsa, Lendrum’s phloxine tartrazine, periodic acid-Schiff, Ziehl-Neelsen, oil red O, Scharlach R, and Nile blue sulfate with and without preceding acetone extraction. Giesels silver impregnation was used for melanin.

For electron microscopic study blocks of tissue from the substantia nigra and locus caeruleus were post-fixed for 2 hours in osmium tetroxide buffered to pH 7.4 with veronal acetate (12) which contained 0.4 per cent CaCl₂. The tissue was dehydrated in a graded series of cold ethanol solutions (50, 70, 95 and 100 per cent) at such a rate that they reached absolute alcohol within 5 minutes (13). Dehydration was continued in cold absolute alcohol for 30 minutes, then in several changes of absolute alcohol at room temperature for 30 minutes. The specimens were further dehydrated with 2 changes of propylene oxide for 10 minutes each, prior to embedding in Epon 812 (14). Serial 3 μ thick sections were cut on a Porter-Blum microtome, mounted in paraffin oil, and examined with the phase microscope for the presence of nerve cells containing Lewy bodies and melanin. More than 102 Lewy bodies were identified by this method, and were subsequently thin sectioned and placed on formvar coated copper mesh grids. The sections were stained with uranyl acetate for 5 minutes (15) and lead citrate (16) for 30 minutes before examination. Electron micrographs were taken with a Siemens Elmiskop I.

CASE REPORTS

Case 1. History: The patient was a 59 year old man who developed symptoms of Parkinson’s disease beginning 13 years before death. At the onset of his disease he had slowness of movements, paucity of spontaneous motion, loss of associated movements, stooped posture and generalized rigidity. He subsequently developed extreme flexion of the trunk, tremor of the right upper extremity, a monotonous low amplitude speech, hypersalivation, and propulsion. About 5 months before death an enlarged supravacuicular node was removed and diagnosed as Hodgkin’s disease. In spite of x-ray treatment and decrease in size of lymph nodes, he expired suddenly after a 4 weeks’ hospitalization.

Post Mortem Findings: At autopsy the gross examination of the brain showed only a minor decrease in width of the cerebral gyri. Microscopically, there was a slight decrease of nerve cells in the globus pallidus. The major changes were in the midbrain where there was a moderate decrease in the number of nerve cells and melanin in the substantia nigra, a mild proliferation of astrocytes, and a considerable number of Lewy bodies within nerve cells and, occasionally, extracellularly. Lewy bodies were also present in the locus caeruleus. The Lewy bodies were varying shades of pink when stained with hematoxylin and eosin, Giemsa, and Lendrum’s phloxine tartrazine; they were non-acid-fast, and were unstained with periodic acid-Schiff and oil red O. Melanin was black with Giesels silver impregnation, brown with hematoxylin and eosin stain and with the Ziehl-Neelson stain, but was reddish brown with the periodic acid-Schiff, oil red O and Scharlach R stains. Melanin appeared green with Nile blue sulfate stain, but had a golden color when this stain was used after acetone extraction. Lipofuscin was red or red-orange when stained with periodic acid-Schiff, Ziehl-Neelson, oil red O, and Scharlach R stains.

Case 2. History: The patient was first seen by a neurologist 10 years before death at the age of 62 years because of involuntary movements of the left hand. There was a past history of hyperthyroidism, angina, and of the symptoms and signs of a myocardial infarct one year previously.

Examination: Neurological examination revealed tremor of the left hand and later of the left leg. Eight years prior to death the patient had bilateral, “pill-rolling” tremors at rest, rigidity and limitation of upward gaze. A diagnosis of Parkinson’s disease was made. There was no mental deterioration, but the patient showed continued progression of his disease with marked difficulty in walking, festination, retropulsion, and micrographia, and died ten years after the onset of the neurological signs.
**Fig. 1.** A nerve cell from the locus caeruleus of Case 2 contains 2 Lewy bodies having moderately dense relatively homogeneous centers and less dense peripheral zones. Melanin granules surround the bodies and separate them from the eccentric nucleus. Phase photomicrograph; ×1,000.

**Fig. 2.** A nerve cell from the substantia nigra of Case 1 in which 2 Lewy bodies having moderately dense centers have joined to form a single elongated structure. A less dense
**Observations of Lewy Bodies and Melanin Granules**

*Post Mortem Findings:* Gross examination of the brain at autopsy showed no abnormalities except for a severe decrease in pigmentation in the substantia nigra, bilaterally; only a small amount of pigment was present in the medial portion of the substantia nigra. Microscopic examination revealed a marked decrease in the number of nerve cells and melanin in the substantia nigra particularly in its lateral two-thirds, and many of the nerve cells remaining were in various stages of disintegration. Small aggregates of extracellular melanin were numerous, and there was a slight proliferation of astrocytes. A few Lewy bodies were present in the nerve cells of the substantia nigra and locus caeruleus. The Lewy bodies, melanin, and lipofuscin showed the same staining characteristics as those in Case 1.

**RESULTS**

*Phase Contrast Microscopy:* The 102 Lewy bodies which were studied by phase contrast microscopy exhibited many morphologic variations, which were similar to those seen by light microscopy. Nerve cells containing 1 to 4 Lewy bodies were seen, usually surrounded by melanin granules. The body commonly consisted of a moderately dense relatively homogeneous center surrounded by a less dense zone (fig. 1). We refer to this form of inclusion as a simple Lewy body. Occasionally, adjacent Lewy bodies so closely approximated one another that they appeared as a single elongated structure (fig. 2). In this instance the less dense zones joined to form the central part of the body. Where a very clear halo existed between the less dense zone and the melanin granules, there was probably an artifactual retraction of tissue.

More complex Lewy bodies were multilaminated and showed variations of their central density. Some had a single dense lamina between a less dense core and the peripheral zone (fig. 3). Others had a dense core, surrounded by multiple concentric laminae of alternating densities (fig. 4). Extreme density of the core might also be encountered in multilaminated bodies (fig. 5). An occasional Lewy body was seen which in serial sections appeared to be separated from a neural perikaryon (fig. 6).

*Electron Microscopy:* The Lewy body in its simplest form consisted of an inner core, which appeared moderately dense, and an outer zone composed of radially oriented filaments (fig. 7, arrow). There was no limiting membrane separating the filaments from the surrounding melanin granules (MG), and, in places, the filaments extended out between the granules. At higher magnifi-

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*Fig. 3.* A moderately dense Lewy body from the substantia nigra of Case 1 has a dense lamina between the core and the peripheral zone. The nucleus is eccentrically located in the granular cytoplasm, which contains a few melanin granules. Phase photomicrograph; × 1,000.

*Fig. 4.* A multilaminated Lewy body within a nerve cell of the substantia nigra of Case 1 contains a dense center surrounded by multiple concentric laminae of alternating densities. Abundant melanin granules are present. Phase photomicrograph; × 1,000.

*Fig. 5.* Two Lewy bodies from the locus caeruleus of Case 2, have laminae of alternating densities surrounding a very dense central core. The cytoplasmic artifact between the Lewy bodies did not interrupt the continuity of the nerve cell in serial sections. Phase photomicrograph; × 1,000.

*Fig. 6.* Serial sections show that this moderately dense laminated Lewy body from the substantia nigra of Case 1 is not located within a neuron. Phase photomicrograph; × 1,000.
Fig. 7. An electron micrograph of a section through the central portion of the 2 Lewy bodies seen in Figure 1, and through a more peripheral region of a third. The moderately dense central regions appear homogeneous at this magnification. Radially oriented filaments (arrow) in the peripheral portions of the inclusions correspond to the outer less dense zones seen in the phase micrograph. Lobulated melanin granules (MG) having vacuole-like structures are present in the cytoplasm; × 8,500. A higher magnification of a serial section (inset) shows very little dense material in many melanin granules (crossed arrow), but some contain the usual amount; × 13,500.
cation one could distinguish closely packed linear profiles about 70 to 80 Å in
diameter in the core (fig. 8, arrow), and profiles of the same width forming
rings about 400 to 600 Å in diameter (crossed arrow and inset). In the outer
zone the filaments (F) were irregular and varied in diameter from 75 to 200 Å,
and were more widely spaced.

When multiple Lewy bodies in a cell closely approximated each other, the
peripheral filaments might be enmeshed producing a greater density and cre-
ating an apparent fusion (fig. 9, X) of the bodies into a single elongated
structure. Within the junctional zone the filaments (fig. 10, F) were more
closely packed and in some places appeared altered, forming a more homogene-
ous granular mass (X).

The concentrically laminated Lewy body might have a core of the same
density and structure as the simple Lewy body, or the core might be much
denser (fig. 11). A fine granularity could, nevertheless, be distinguished within
these extremely dense cores (fig. 12, C). The periphery of the core had an ir-
regular outline where it merged with the surrounding zone.

The laminae within Lewy bodies were formed by variations in the density
and arrangement of filaments. In those laminae which were slightly dense, the
filaments were somewhat more closely packed (fig. 13, arrows). Denser laminae
(fig. 11, arrow and fig. 14) had an even larger number of filaments and a
greater variation in their direction. Some circular (fig. 14, crossed arrow) and
elongate profiles might be present which were identical to those seen within
the core of the simple Lewy body.

The melanin granules within the neurons of the substantia nigra of the
Parkinsonian cases studied were irregularly shaped, lobulated complex bodies
containing vacuole-like structures, and a variable amount of dense material
(fig. 7, MG, and inset). At higher magnification some of these melanin gran-
ules were seen to be filled with aggregates of very dense irregularly shaped
particulate material, which had indefinite boundaries (fig. 15, arrow). In
other melanin granules the very dense particulate material (fig. 16, arrow)
was sparse, revealing a substructure of moderately dense linear (crossed arrow)
and punctate arrays of about 70 to 80 Å in diameter. These appeared much
smaller in size than the very dense particulate material, although the size of
the latter is only approximate, because of its indefinite boundaries and tend-
ency to clump.

Melanin granules within the neurons of the substantia nigra of normal brains
had a lobulated configuration and contained protruding vacuole-like struc-
tures (fig. 17, inset). The granules were filled with small, very dense, irregu-
larly shaped, particulate material, which had indefinite boundaries (fig. 17,
arrow). In limited areas in which the very dense material was less abundant, a
granular or linear substructure was present (crossed arrow). Although gran-
ules might be seen in which the very dense material was as sparse as in melanin
in nerve cells of cases of Parkinson's disease, this was rare.

The fine structure of lipofuscin granules within the neurons of pontine nu-
clei of normal brains was compared with melanin granules from normal and
Fig. 8. A higher magnification of the junction of the center and the peripheral filaments shown at arrow in Figure 7. The core of the Lewy body is composed of elongate (arrow) and circular profiles (crossed arrow). The outer zone contains more widely spaced irregular filaments (F); × 86,000. Circular and elongated profiles are shown at higher magnification in the inset; × 132,000.
Fig. 9. An electron micrograph of a section through the Lewy bodies seen in Figure 2. An increased density is apparent at the junction (X) of the 2 bodies. The radial orientation of the peripheral filaments is evident; × 10,200.

Fig. 10. A higher magnification of the junction of the 2 Lewy bodies seen in Figure 9. In one area the filaments (F) are closely packed, but in another they appear altered forming a more homogeneous granular mass (X); × 68,000.
Fig. 11. An electron micrograph of a section through the rounded Lewy body seen in Figure 5. The core appears very dense. A pronounced second lamina (arrow) is formed of closely packed filamentous material, whereas the laminae on either side of this are less densely packed. The peripheral filaments are radially oriented; × 14,000.

Fig. 12. The dense core (C) of the Lewy body in Figure 11 is finely granular. Filaments (F) of the surrounding lighter lamina merge with the granular center forming an irregular junction; × 144,000.
Fig. 13. The moderately dense lamina of the Lewy body in Figure 6 shows only a somewhat closer packing of filaments (arrows) than the zones on either side; $\times 25,300$.

Parkinsonian brains. The lipofuscin granules had a lobulated configuration and contained protruding vacuole-like structures (fig. 18, inset). The internal structure was composed of moderately dense, punctate and linear arrays (fig. 18, crossed arrow) of about 70 to 80 Å in diameter.

COMMENT

The Lewy body is a spherical inclusion which has a central core surrounded by a single less dense zone or by laminae of alternating density. Cytologic details and staining reactions were described by Greenfield and Bosanquet (5). A number of these stains were used to confirm the identity of the inclusions observed in the present study. A direct correlation of the phase image and the fine structure of the Lewy body was possible in this study because the inclusions identified by phase microscopy were serially sectioned for electron microscopic observation.

The electron microscopic observations demonstrate that the Lewy body is composed of a grouping of filamentous structures. The different appearances of this inclusion as seen by light and phase contrast microscopy are accounted for by the variations in number and arrangement of these filaments and by an alteration in their structure.

The less dense outer zones, as seen by phase microscopy (fig. 1), are due to a loose packing of radially oriented filaments (fig. 7). Very clear halos, sometimes seen beyond the less dense outer zone (fig. 2), are probably due to an artifactual retraction of the tissue. The density of alternating laminae (figs. 3, 4, 5, 6) is due to the number and closeness of packing of the filaments; the
Fig. 14. The very dense lamina seen in the Lewy body at arrow in Figure 11 extends obliquely across the micrograph. It has a larger number of filaments and a greater variation in their direction than the less dense zones on either side. Some circular (crossed-arrow) and elongate profiles are present in the very dense lamina. Part of the dense core (C) and the filaments (F) of the outer zone are present; × 66,000.

denser the lamina the greater the number and disarray of filaments (figs. 13, 14).

The core as seen by phase microscopy (fig. 1) also has a dense packing of filamentous material, which usually consists of circular and elongated profiles (fig. 8, and inset). Increased density of the center (fig. 5) appears to be due to an alteration in the filamentous material to form a homogeneous granular mass (figs. 11, 12).

The coalescence of the peripheral filaments of two adjacent Lewy bodies (figs. 9, 10) accounts for the oblong-shape of some Lewy bodies, as seen in light or phase microscopy (fig. 2).
Fig. 15. A melanin granule from the locus caeruleus of Case 2 is filled with aggregates of very dense irregularly shaped particulate material, having indefinite borders (arrow). Vacuole-like structures and a small portion of the granular substructure (crossed-arrow) can be seen; × 132,000.

Fig. 16. A melanin granule from the locus caeruleus of Case 2 contains only a few aggregates of the very dense irregularly shaped particulate material (arrow). The remainder of the granule is composed of granular and linear material (crossed-arrow) of moderate density; × 132,000.
Fig. 17. A typical melanin granule of the substantia nigra from a normal brain appears dense and lobulated (inset); $\times$ 20,000. Vacuole-like structures, that appear clear or have a moderate density, protrude at the surface or are within the body. At higher magnification the granule is seen to contain aggregates of small very dense irregularly shaped particulate material, which has indistinct borders (arrow). Only a small portion of the granular substructure (crossed-arrow) may be seen; $\times$ 132,000.

The fine structure of Lewy bodies is unlike that of corpora amylaceae of the brain as described by Ramsey (17, 18) and as seen in this material. The radial arrangement of filaments and their greater length in the Lewy body (figs. 7, 8) contrast with the disorderly array of short linear structures and small osmiophilic bodies in corpora amylaceae (17, 18). Lewy bodies also differ in the presence of circular and elongated profiles (fig. 8, and inset) or by a homogeneous granular appearance centrally (fig. 12). Moreover, Lewy bodies are usually found in neural perikarya, whereas corpora amylaceae are reported to be within the fibers of fibrillary astrocytes (17, 18).

Closely packed filamentous structures are the major components of neurofibrillary tangles and senile plaques (19, 20) as well as of Lewy bodies. Neurofibrillary tangles, as reported by Terry et al (19, 20) in Alzheimer's presenile dementia, are composed of an increased number of intracellular neurofibrils, which are otherwise normal. These fibrils occur in bundles, but do not interweave with one another. The center of the senile plaque, on the other hand, is
made up of a different sort of filamentous material, which is extracellularly located and structurally identical with amyloid. Hollow filaments are interwoven into bundles, which generally extend peripherally from the center (19, 20). Since the Lewy body is also composed of filamentous structures, one may consider whether neurofilaments contribute to its formation, as they do to neurofibrillary tangles (19, 20). If this is the case, there are significant differences in the arrangement and possibly in the nature of the filaments. Aside from the radial orientation of filaments in the Lewy body, the presence of circular profiles and their apparent fusion into a granular mass would represent an alteration of neurofilaments not seen in neurofibrillary tangles (19, 20).

Remnants of the melanin granule itself may contribute to the formation of the Lewy body, since large numbers of these granules in various stages of
breakdown fill the cytoplasm of cells having Lewy bodies (fig. 7 and inset). This consideration is supported by the finding of linear components in the substructure of altered melanin granules (fig. 16, crossed arrow). Moreover, the diameter of these linear arrays is of the same order of magnitude as the circular and elongate profiles within the core of Lewy bodies (fig. 8, inset).

Although altered neurofilaments and portions of melanin granules have been suggested by these electron microscopic observations as possible components of the Lewy body, other sources have not been excluded. The Lewy body, therefore, represents a type of filamentous degeneration within neurons, but the origin of the filaments requires further clarification.

The normal melanin granule is a complex body which includes a very dense component, vacuole-like structures, and a substructure (fig. 17). Many melanin granules within neurons of Parkinsonian cases (fig. 15) cannot be distinguished from the normal. In abnormal melanin of Parkinsonian cases, however, the substructure is more apparent (fig. 16) because there is often a decrease in the very dense component. The substructure including the presence of linear arrays resembles lipofuscin in normal nerve cells of pontine nuclei (fig. 18). Linear densities of indeterminable length were previously described in lipofuscin granules of cortical neurons by Gonatas et al. (21), but were not present in another case (11). Lipofuscins are often considered to be a lipoprotein complex (22). The vacuole-like structures of lipofuscin have been thought to be lipid, whereas lysosomal enzymes have been reported in the remainder of the granule (23). Since one cannot distinguish any morphological differences between the substructure of the melanin granule and that of lipofuscin, one must consider whether the melanin granule might be composed of a lipofuscin substructure upon which a very dense material, the melanin pigment-complex, is deposited. The substructure might correspond to the premelanosome of other tissues (24). Whether the substructure of the melanin granule is in fact a type of lipofuscin containing a melanin forming enzyme or whether there is a chemical difference must be clarified by other techniques. Pigmented neurons are decreased in number in the substantia nigra and locus caeruleus in the brains of cases of Parkinson's disease, but it has not been known whether the melanin decreases because of the breakdown of the cells or whether some alteration in the melanin granules occurs before the complete disintegration of the neuron. The observation of a decrease in the dense component of melanin before the complete breakdown of the substructure or of the neuron itself may have an important bearing on the mechanism of depigmentation in nerve cells in this disease.

SUMMARY

The fine structure of Lewy bodies and melanin in 2 cases of Parkinson's disease is described and compared with their phase and light microscopic appearance. The Lewy body is composed of a group of filamentous structures. In the less dense outer zones of this body the filaments are radially oriented and loosely packed. The denser laminae are the result of a closer packing of
more numerous filaments. The moderately dense cores are formed of circular and elongate profiles. Dense cores appear to be due to an alteration in the filamentous material to form a homogeneously granular mass. Adjacent Lewy bodies may coalesce to form an oblong structure.

There is often a decrease in the very dense component of the melanin granule within neurons of cases of Parkinson's disease. The substructure of the altered melanin granules resembles that of lipofuscin.

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Addendum: Since this paper was submitted for publication a study, comparing pigment in nerve cells with melanin in other tissues, has appeared. Moses, H. L., Beaver, D. L., and Ganote, C. E.: Light and Electron Microscopic Studies of Pigment in the Substantia Nigra and Locus Coeruleus. Anat. Rec., 151: 391, 1965. (Abs.).

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