133. *Electron Microscopic Study of Pigment Formation in the Human Substantia Nigra*

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Introduction. The presence of dark brown pigment granules, termed neuromelanin by Lillie,¹⁾ in the neurons of the substantia nigra and locus caeruleus has been recognized.²⁾ In recent years, evidence has been present which suggests that the neuromelanin may not be formed through the tyrosine-tyrosinase system of cutaneous melanin^{3),4)} and that the neuromelanin may differ in chemical composition from extra-neural melanin.⁵⁾ In addition, ultrastructural differences between them have been reported by several investigators.⁶⁾⁻⁸⁾ However, the mode of formation of the neuromelanin granules is still unknown. Therefore, in an attempt to investigate the pigment formation an electron microscopic study of the human substantia nigra was initiated.

Material and method. The substantia nigra from 10 human materials (infants of 1, 6 months, 1, 3, 4, 5 years old and adults of 58, 62 years old; two cases for each of 4-, 5-year-old infants) was fixed in 10% neutral formalin for a considerable period and then fixed in osmium tetroxide phosphate-buffered at pH 7.4 followed by dehydration and embedding in Epon 812.⁹⁾ Thin sections for electron microscopy were cut with glass knives on a Porter-Blum microtome, were doubly stained with uranyl acetate and lead acetate¹⁰⁾ and then examined with a HU-11A electron microscope.

Observation. Ultrastructurally, the mature neuromelanin granules were composed of three distinct components. The first was a finely particulate, medium-dense matrix which showed occasional linear configurations similar to those of lipofuscin granules.¹¹⁾ The fine particles were round or rod-shaped and about 60 to 80 Å in diameter. The second was a very dense, coarsely particulate material, which was not surrounded by any limiting membrane. The coarse particles were round or rod-shaped and about 100 Å in diameter occasionally exhibiting indistinct linear arrays. The third was a membrane-bounded electron-lucent vacuole which is presumably a lipid globule. The vacuole was often found in contact with the above mentioned dense material.

Within nigral neurons of three-year-old children, were often

Fig. 1. A lysosomal dense body within a nigral neuron of a 3-year-old child. Note the extremely electron dense particles (arrows) randomly deposited in the matrix. $\times 76,800$.

Fig. 2. Triphasic neuromelanin granules within a nigral neuron of a 4-year-old child. Lipid globules (L) are bounded by a limiting membrane. $\times 42,000$.

Fig. 3. A triphasic neuromelanin granule of a 5-year-old child. Note an increase in the number of the extremely dense particles. $\times 41,300$.

Fig. 4. Portion of an irregularly-shaped mature neuromelanin granule of an adult showing three structural components. $\times 22,800$.

found lysosomal dense bodies, on the matrix of which a few extremely dense particles were found deposited singly or in clusters (Fig. 1). Triphasic pigment granules were few in number and small in size. Melanosome-like structures could not be recognized.

In the case of a 4-year-old child, the lysosomal dense bodies were few in number and the triphasic pigment granules increased in number and size (Fig. 2).

In the case of 5-year-old children, increase in the number of the electron dense, coarse particles was striking (Fig. 3). Triphasic granules were frequently found to coalesce with each other. The limiting membrane of the granules were occasionally detected, but in their matrix, lamellar structures were only rarely observed.

In adults, all pigment granules were triphasic and remarkably increased in number, but, their size and shape varied considerably in different granules. Their limiting membrane was not always detectable (Fig. 4).

Ultrastructurally, the pigment granules of the substantia nigra differed from those of the pia mater in that the latter were homo-

geneously dense ellipsoidal small granules. Lipid globules were not observable in the pial melanin granules.

Conclusion. 1. The formation of the neuromelanin granules begins in the form of the lysosomal dense bodies, on the matrix of which a dense particles were found deposited singly or in clusters.

2. The formation of the second component, a very dense, coarsely particulate material, were followed by the formation of the third component, a membrane-bounded, electron-lucent vacuole.

3. Triphasic pigment granules were few in number and small in size in the substantia nigra of the 3-year-old children. Melanosome-like structures cannot be recognized.

4. Ultrastructurally, the pigment granules of the substantia nigra differed from those of the pia mater.

5. Lipid globules were not observable in the pial melanin granules.

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