EFFECTS OF PROLONGED LSD-25 ADMINISTRATION UPON NEURONS OF SPINAL CORD GANGLIA TISSUE CULTURES

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ABSTRACT

Rat spinal cord ganglia cultures were maintained for periods of up to 19 days in a feeding solution containing LSD-25 in a concentration of 5×10^{-6}. Electron microscopic examination of the nerve cells in these cultures revealed alterations in the Golgi complexes, lysosomes, mitochondria and multivesicular bodies. The changes in the membranous components of these structures were particularly prominent and resulted in organelle pleomorphism and very unusual internal membrane patterns. Variations were also noted in the fine structure of the nucleoli of some neurons. The possible relationships of the morphologic changes to disturbances of neuronal metabolism are considered.

INTRODUCTION

LSD-25 (lysergic acid diethylamide-25) is one of the most commonly used and abused hallucinogens (1, 37). Since the biochemical and pharmacological properties of this psychedelic drug have been established (2, 7, 14, 27) special attention has now focused upon its behavioral (9, 14, 24) and possible teratogenic (4, 5, 10, 12, 17, 21, 32, 35, 40) effects as well as its interaction with other drugs such as the narcotics and alcohol (33).

Relatively few studies have considered its adverse or toxic effects upon the structural constituents of the CNS either in vivo (12, 25) or in vitro (18, 26). Recently Hendelman (20) reported on the electron microscopic changes induced by the drug in organotypic cultures of newborn mouse cerebellum. In this study, cultures were exposed for periods of up to 53 hours to a nutrient medium containing LSD. The principal alteration observed was the development of so-called "heterogeneous dense bodies" (HDBs) which were felt to be of lysosomal origin.

The present investigation is designed to study the effects of LSD-25 on the ultrastructure of neurons in rat spinal cord ganglia cultures continuously exposed to the drug for prolonged periods of time. The chronic effects of this agent

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on tissues are of particular importance in light of the clinical pattern of repetitive LSD usage observed in some individuals, as well as the recurrence of psychedelic phenomena (flashbacks) long after the drug has been discontinued.

MATERIALS AND METHODS

LSD: Delysid (LSD-25), Sandoz Pharmaceuticals, was used in a concentration of 5×10^{-6}. Before it was mixed with the feeding solution, purity was tested by spectrophotometry. The drug was dissolved in a feeding solution, containing 33% cord serum, 54% minimum essential medium (Eagle), 10% mouse embryo extract and 3% glucose. The feeding solution was stored at 2°C and was freshly prepared every 30 days. The sister cultures of the same batch were used as controls and were fed with an identical feeding solution, minus LSD.

Cultures: Rat spinal cord and dorsal root ganglia cultures, representing central and peripheral nervous system, were prepared from 13 day old fetuses. The fragments, approximately 1 mg. of tissue, were explanted on collagen-coated coverslips and mounted in a Maximow chamber. The cultures were fed every third day. Light microscopic observations, at magnifications of 125–500×, were done every second day. Cultures from which the feeding solution had drained were discarded.

Electron microscopic procedures were carried out in the following manner. The cultures were grown under circular glass coverslips, diameter 18 mm. These were placed in Columbia dishes and rinsed three times with Simms' balanced salt solution to remove the protein containing culture medium. Immediately after the final rinse the dishes were filled with one of the following fixatives: A) Palade's buffered osmium tetroxide fixative or B) Millonig's buffered osmium tetroxide. The fixation lasted for 1 ½ hours. After fixation, the cultures were rinsed with the same buffered solution as used in the fixative. The coverslips remained in the Columbia dishes during ethanol dehydration and the initial phases of Epon infiltration which followed Luft's method. After overnight soaking in the Epon 812 mixture at room temperature, the coverslips were removed from the Columbia dishes by a modified Bunge method (8). The sections were cut on the LKB ultratome 8801A with a Dupont diamond knife at 600–700A° thickness. The sections were collected on 300-mesh specimen grids and stained with uranyl acetate and lead citrate, according to Watson's method. The electron microscopic examinations were carried out with RCA EMU-3G electron microscope. Since it is known that degenerating cells are commonly encountered in certain regions of the explant, a special effort was made to exclude these zones from the study. Because of difficulty in accurately identifying all of the cultured elements, observations were limited to those cells which could be identified as neurons.

Light Microscopy

The earliest changes at a light microscopic level of resolution were seen on 10–11th day after exposure to LSD. They consisted of a mild to moderate granularity of glial and connective tissue elements. No unusual features were observed in the neurons, Schwann or satellite cells. The prominence of the granularity in the affected cells increased during subsequent days and by the 18–19th day a slight granularity was also seen in some neurons. Since it is known that nonspecific degenerative changes often occur in the peripheral portion of the outgrowth the changes in this region were disregarded.

Electron Microscopy

Control Cultures—11 days in vitro (DIV): The ultrastructural features of the control culture neurons were generally similar to those described by pre-

vious investigators (8). The appearance and extent of the endoplasmic reticulum and Golgi complex showed average variations as did the distribution and fine morphology of the cytoplasmic organelles. The nucleolus was usually single and in most instances centrally located. In a few cells, however, it was eccentric and aligned with or attached to the nuclear membrane. Some cells had multiple nucleoli. The "pars granulosa" and "pars fibrosa" were usually well differentiated but the "pars chromosa" was less clearly defined. A few necrobiotic areas were seen.

LSD Cultures—11 days in vitro (DIV): Some nerve cells in the cultures exposed to LSD resembled the control neurons but many showed qualitative and quantitative ultrastructural alterations. The extent and distribution of the endoplasmic reticulum was somewhat more variable than in the controls. A few cisternae of the rough portion of the endoplasmic reticulum were dilated and contained a homogenous substance of low electron density. Some of the Golgi canaliculi were irregularly dilated, and numerous saccules, vacuoles and vesicles were seen in the perikaryon.

The mitochondria (pleomorphic metabolosomes) were sometimes clustered, usually in the cell body (Fig. 1), but occasionally in a neurite. Many of these organelles had unusual shapes, and some were gigantic. The pattern of arrangement of the cristae mitochondrialis was frequently disorganized and matrix density was somewhat variable.

The lysosomes showed even more variability in regards to number, form and osmiophilia (Fig. 2). Some had limiting-membrane alterations such as differences in thickness and osmiophilia. Many contained membranous arrays which varied in appearance from very simple forms occupying only a small portion of the organelle (Fig. 3A–E) to very complex structures almost completely filling it (Fig. 3F–H). Many lysosomes resembled the "heterogeneous bodies" described by Hendelman (29).

Centrioles and multivesicular bodies (MVBs) were seen in some cells. The latter varied considerably in number and were sometimes arranged in clusters. They, like the lysosomes, showed membrane alterations and differences in size, shape and matrix density (Fig. 4A and B). Some MVBs contained unusual inclusions, and there was some variability in the number of vesicles (Fig. 4A and B).

Abnormalities were also observed in the fine structure of the nucleus, in particular the nucleolus. The nucleoli of some of the neurons exposed to LSD resembled those seen in the controls but many showed morphologic alterations. In some there was a condensation of the dense granular portion (ribosomal component) of the "pars granulosa" which caused this structure to appear darker and denser than usual. In some instances it was difficult to distinguish "pars granulosa" from the "pars fibrosa" (Fig. 5A). In other cells the granular material of the "pars granulosa" was aggregated into small dense masses which were irregularly scattered within the nucleolus (Fig. 5B). In a few cells some of the nucleolar material was rearranged in the form of a ring (Fig. 5C and D).

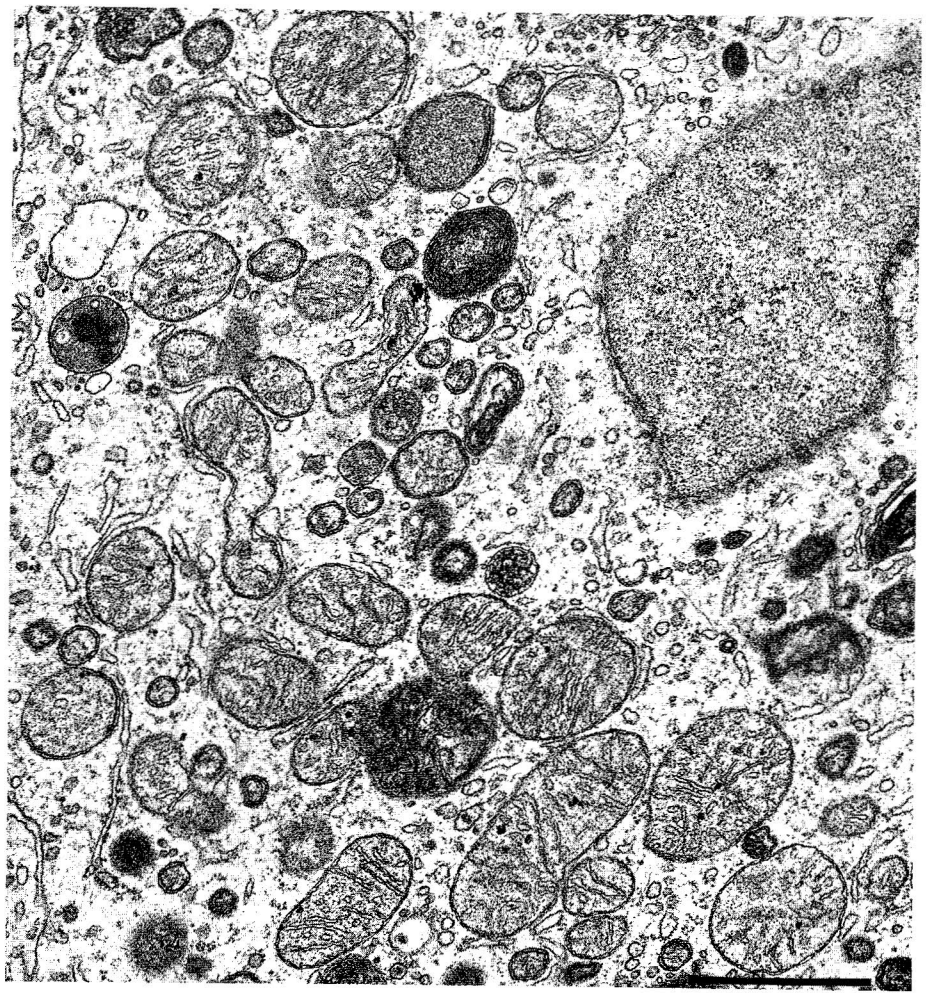

FIG. 1. Segment of an LSD-treated neuron containing increased numbers of organelles, particularly mitochondria. LSD-treated, 11 DIV. × 39,000.

Control Cultures—19 days in vitro (DIV): Although there were some minor differences, the ultrastructural features of the control culture neurons were generally similar to those observed in the 11 DIV control cultures. Mitochondria were occasionally arranged in clusters but their morphology was not unusual. Lysosomes were slightly more varied in number, size, shape and matrix density. A few lysosomes had pseudopode-like projections, and membranous inclusions were observed in some. Several axons contained accumulations of mitochondria, often along with lysosomes and occasionally MVBs. A few necrobiotic areas were observed.

LSD Cultures—19 days in vitro (DIV): The LSD-25 treated cultures showed a number of features not observed in the controls. The most outstanding

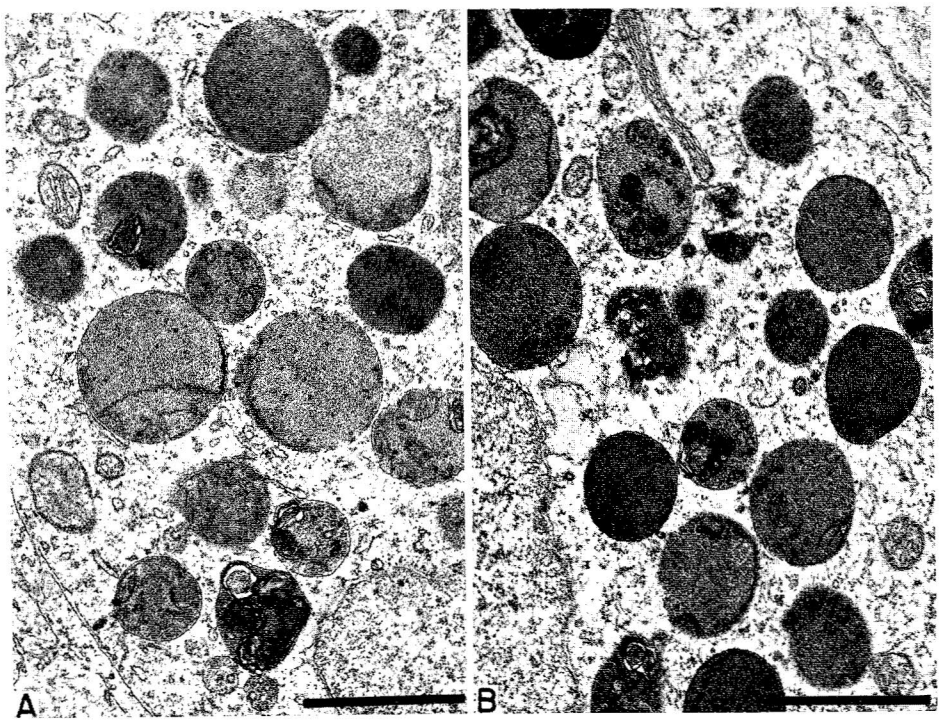

FIG. 2 (A–B). Increased numbers of pleomorphic lysosomes in LSD-treated neurons. LSD-treated, 11 DIV. A × 29,000; B × 29,000.

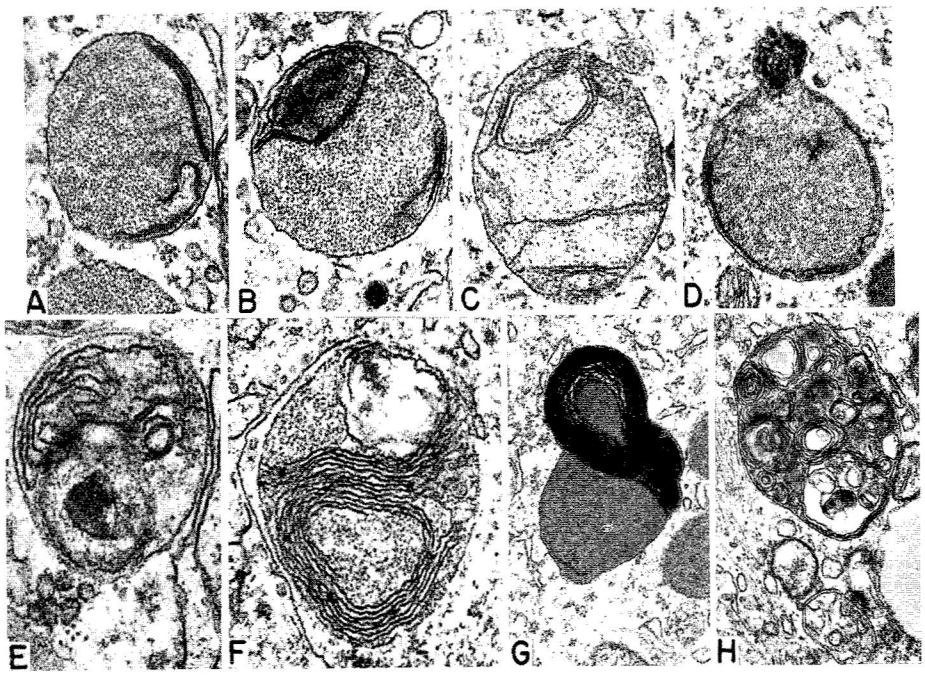

FIG. 3 (A–H). Lysosomes showing fine structural alterations and variations in lysosomal membrane patterns. LSD-treated, 11 DIV. A × 48,500; B × 49,000; C × 63,500; D × 29,000; E × 65,000; F × 63,500; G × 29,500; H × 30,000.

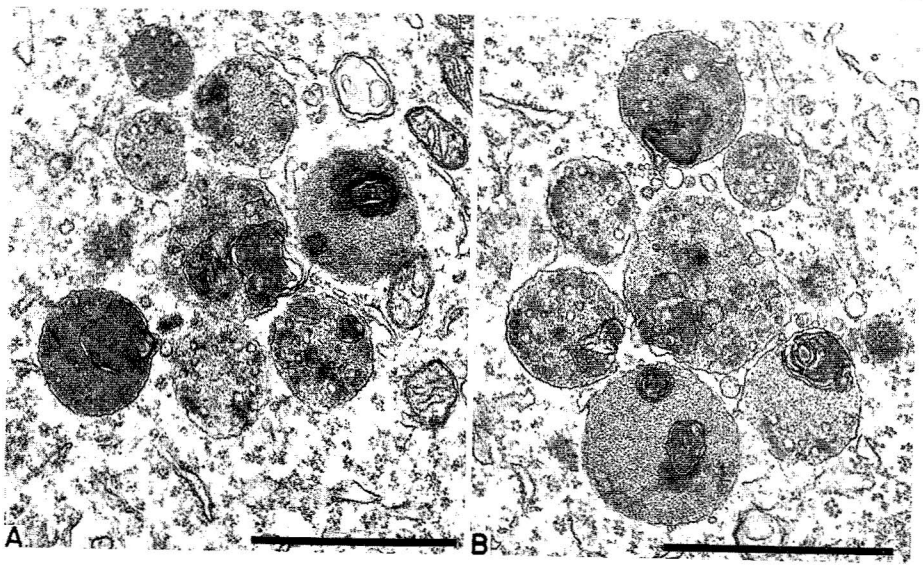

FIG. 4 (A–B). Multivesicular bodies showing both qualitative and quantitative fine structural changes as described in text. LSD-treated, 11 DIV. A × 44,000; B × 48,500.

of these was the presence of large numbers of very unusual mitochondria (Fig. 6). Many mitochondria were oval or irregular, some were elongated or sausage-shaped, others were doughnut-shaped and a few resembled bowling pins in shape. There were marked variations in the internal structure of these organelles, particularly as regards number, size, configuration and arrangement of the cristae mitochondrialis (Fig. 6 and 7). In a few mitochondria as many as four patterns of cristae arrangement were observed (Fig. 7A) and in some the alignment of cristae was quite unusual (7D). In a number of instances the cristae were ribbon-like or had indistinct outlines which blended into the surrounding matrix (7B). The latter was not always completely homogeneous and on occasion contained dense inclusions (7E) or glycogen (7F).

Changes in lysosomes (Fig. 8) similar to those described in the 11 DIV cultures were observed. Some lysosomes contained curvilinear structures (Fig. 9A–D) similar to the curvilinear bodies described by Duffy et al. (16). Although these were observed in some control and in some 11 DIV LSD-treated cultures they were considerably more prominent in the 19 DIV LSD-treated cultures.

Alterations were again observed in the nucleoli. The "pars granulosa" and "pars fibrosa" were often well differentiated, but in many instances the components of the "pars granulosa" were rearranged and formed small homogeneous masses which were scattered within the "pars fibrosa". In some instances the nucleolus was small and dense and the "pars granulosa" could not be differentiated from the "pars fibrosa". Occasionally the "pars granulosa" and "pars fibrosa" were separated (10A and B). A few nucleoli appeared to be disintegrating (Fig. 10C) and their fine structure was not discernible.

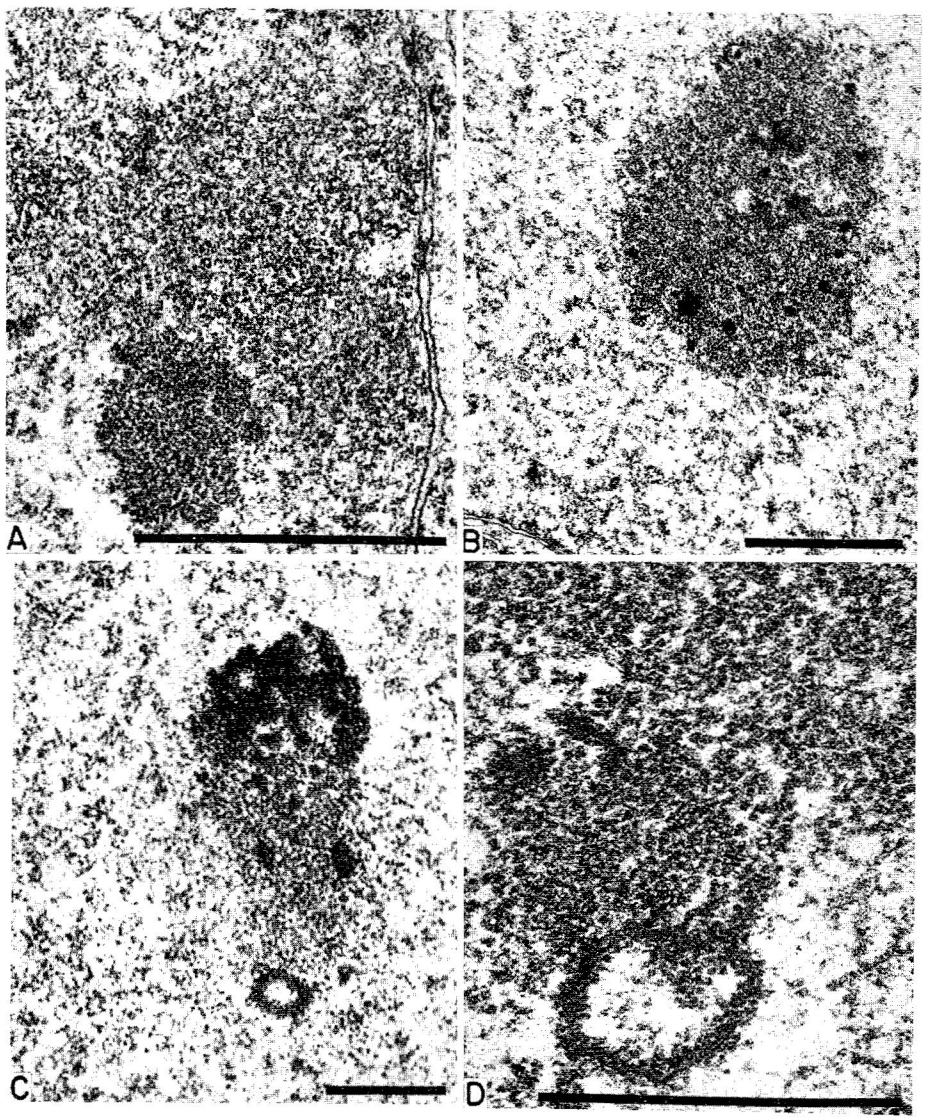

FIG. 5 (A–D). Variations in nucleolar fine structure particularly that of the "pars fibrosa" and "pars granulosa". LSD-treated, 11 DIV. A × 58,000; B × 29,000; C × 22,000; D × 67,000.

DISCUSSION

Light microscopic studies of LSD-treated tissue cultures have shown alterations in cell activity and growth as well as a diminution of Nissl substance (18, 26). Changes have also been observed in the nucleus and nucleolus and prolonged exposure may result in cell death. Hendelman (20) has recently described changes of lysosome ultrastructure in cultured mouse cerebellar neurons exposed to LSD. This author has attempted to explain the "vivid behavioral

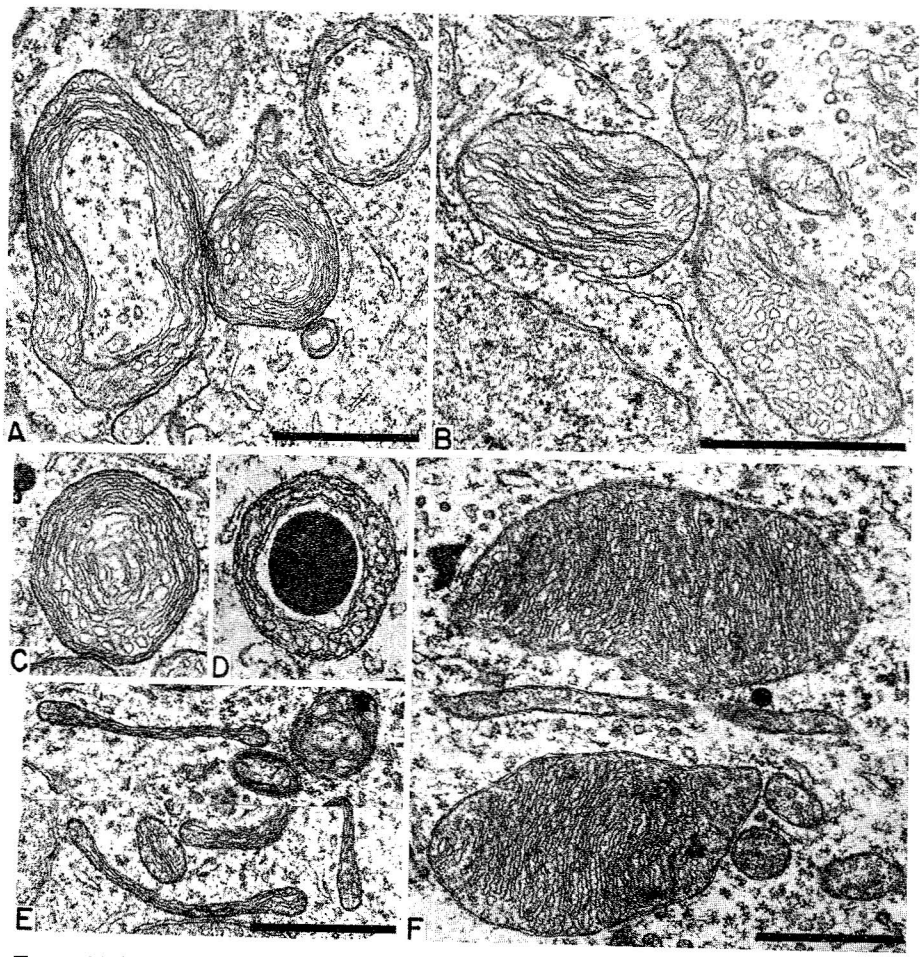

FIG. 6 (A–F). Markedly pleomorphic mitochondria. LSD-treated, 19 DIV. A × 29,000; B × 40,500; C × 40,500; D × 42,000; E × 29,000; F × 29,000.

effects" of LSD in humans on the basis of these lysosome changes. Without entering into a controversy regarding the appropriateness of correlating structural changes in cultured animal tissue with behavioral effects in humans, it seems unlikely on theoretical grounds that changes restricted to lysosomes can be responsible for all of the clinical manifestations produced by LSD.

In the present study, lysosomal alterations similar to those described by Hendelman, including the presence of heterogeneous bodies, were observed. In addition, definite changes were noted in the endoplasmic reticulum, Golgi complex, mitochondria, multivesicular bodies and nucleoli. Some of the most conspicuous of these consisted of abnormalities of the membranous components of cytoplasmic organelles. Since unit membranes are composed of proteo- and phospholipids (11, 29) it seems reasonable to hypothesize that the observed membrane changes might be the result of interference by LSD-25 with interme-

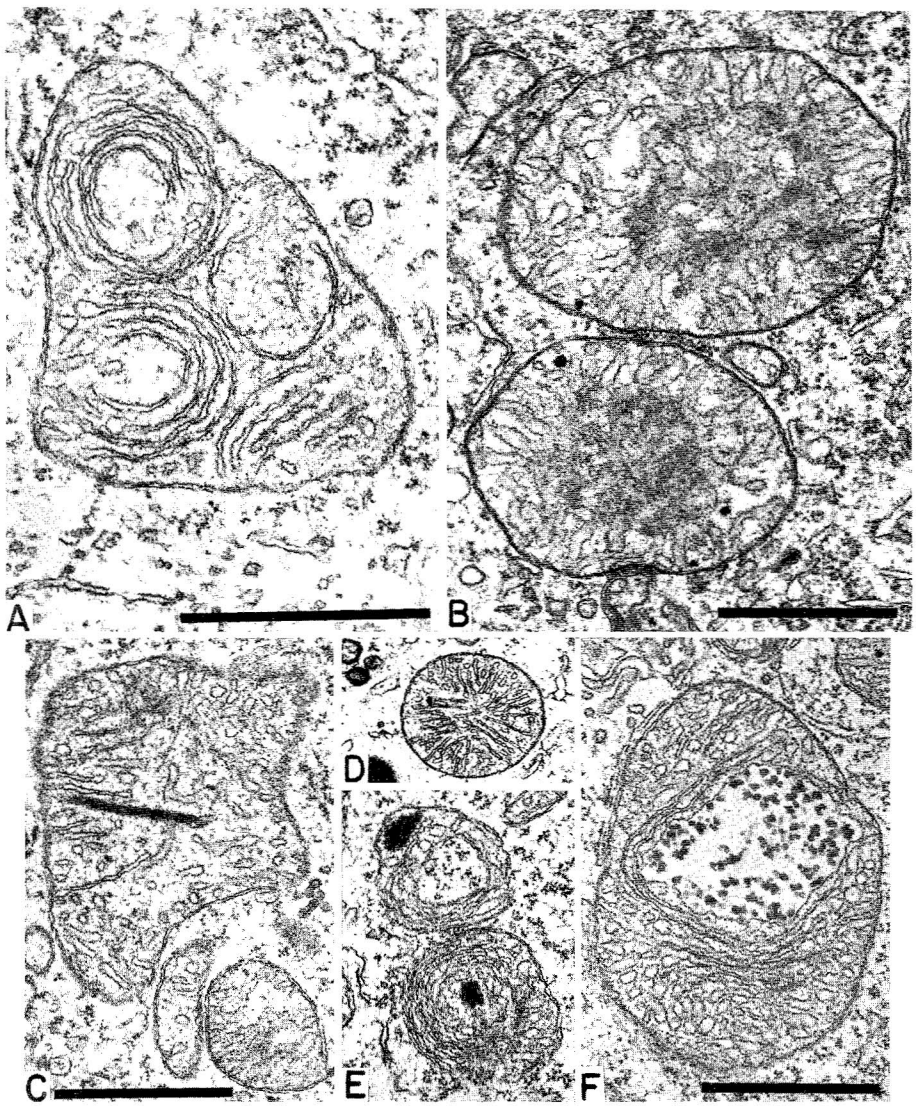

FIG. 7 (A–F). Mitochondria showing marked qualitative and quantitative cristae variations. LSD-treated, 19 DIV. A × 40,500; B × 29,000; C × 29,000; D × 29,000; E × 29,000; F × 29,000.

diate phases of lipid metabolism related to membrane formation. That the morphologic changes observed in the LSD-treated cultures are necessarily connected with neuronal dysfunction cannot be stated with certainty. There is ample evidence, however, that alterations in organelle morphology occur in some disease states and that these changes are an integral part of the pathologic process. In the present context it is of particular interest to note that qualitative and quantitative lysosomal changes have been observed in various

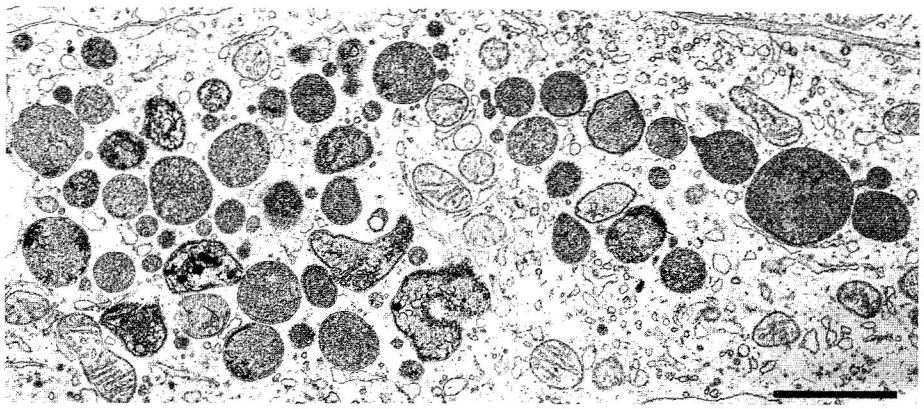

FIG. 8. Lysosomes showing both qualitative and quantitative variations. LSD-treated, 19 DIV. A × 28,000.

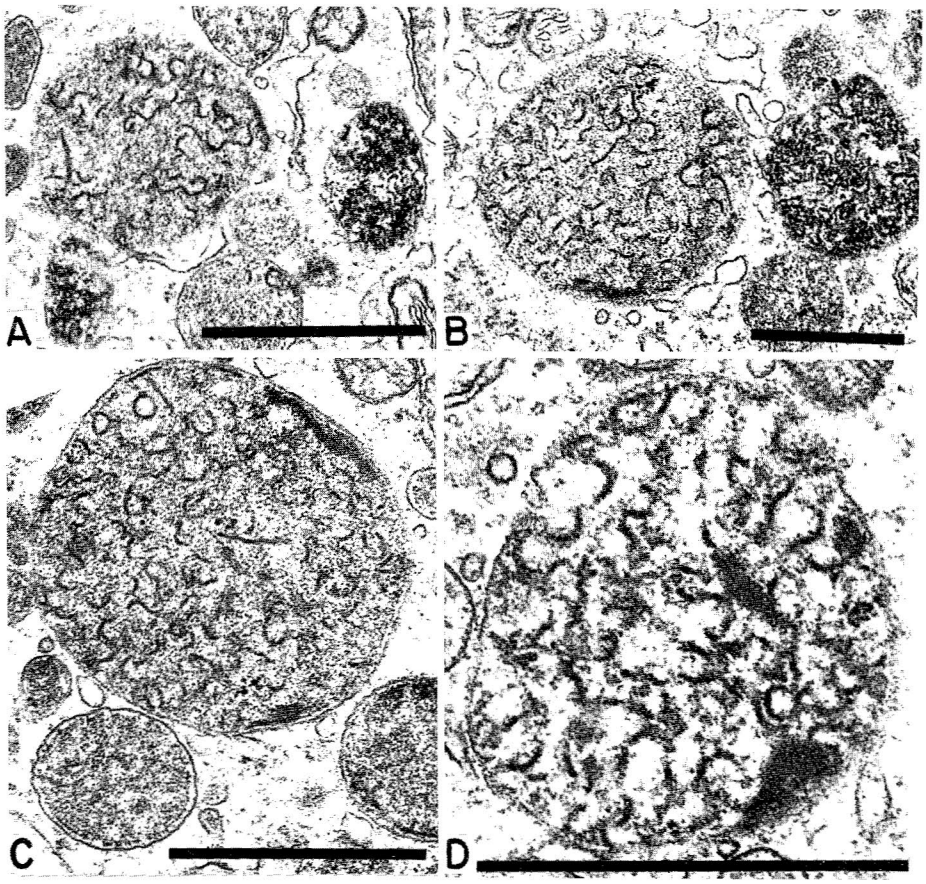

FIG. 9 (A–D). Lysosomes containing curvilinear structures. LSD-treated, 19 DIV. A × 33,000; B × 23,000; C × 38,000; D × 63,500.

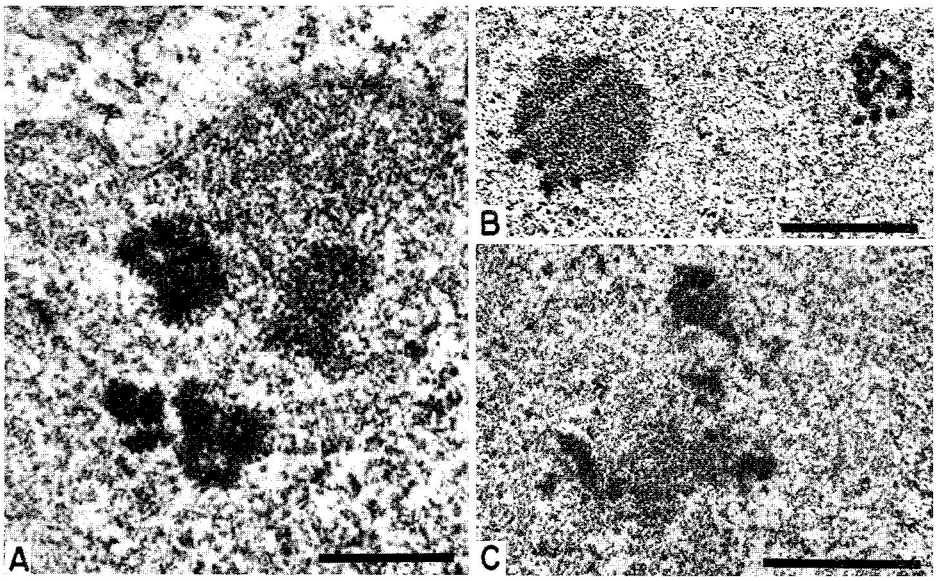

FIG. 10 (A–C). Nucleolar changes, including fragmentation (A and B), as described in text. LSD-treated, 19 DIV. A × 24,000; B × 24,000; C × 29,000.

pathologic processes (6, 23), and that abnormal membranous inclusions have been described particularly in cerebral dyslipidosis (3, 13, 34, 36). It seems likely that the severe structural changes observed in many of the lysosomes in the LSD-treated cultures would be incompatible with normal metabolic activity.

Likewise, the mitochondrial changes seen in our study might be expected to interfere with organelle function. In mitochondria there is an intimate relationship between enzyme systems and membranes. It has been established that certain enzymes are located at specific sites on the mitochondrial membranes (22). It has been also observed that structural alterations of the mitochondria (15, 16, 31) and their membranes are associated with various functional and metabolic disorders (19). It would be expected that marked changes in membrane structure might interfere with certain enzyme-catalyzed reactions, thus disturbing organelle metabolism. However, the extent of this is very difficult to predict.

Morphologic changes were also observed in MVBs. Little is known about the specific function and metabolism of these organelles, but it is believed that they are related to the Golgi complex and lysosomes (28, 30) and that they may participate in endocytosis (28). Whether or not the changes observed in the structure of the MVBs or the minor variations observed in the endoplasmic reticulum and Golgi complex are of functional significance is uncertain, although the possibility must be considered.

Finally, changes were observed in nuclear morphology, specifically in the nucleolus. Although these nuclear alterations may represent nonspecific changes

in degenerating cells, one must also keep in mind that there is other evidence which suggests that LSD does act on certain nuclear components (5, 10, 21, 35). Several investigators have attributed teratogenic effects to LSD-25 (4, 5, 10, 17, 32), and chemical studies have shown that DNA has a strong affinity for and tends to bind with LSD (38). Unfortunately, chromosomal structure could not be studied with the methods employed in this investigation. In any case, it does seem essential that the actions of LSD on nucleic acids must be included in any consideration of the effects of LSD on growing neurons.

Although our electron microscopic studies of the effects of LSD were limited to growing neurons in 11 and 19 DIV cultures, they do give some insight into the complex activity of the drug. The effects of LSD on cytoplasmic organelles, more specifically the structure of their unit membranes, and its possible effects on nucleic acids seem to be of importance and deserve further study. No claim can be made at this stage of our investigations that the changes which were observed in the cultured neurons are specific for LSD, but we are presently trying to clarify this by carrying out similar studies using a variety of other chemical agents.

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