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Original Investigations

Morphological Changes in CNS of Rats Treated with Perhexiline Maleate (Pexid)

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Summary. The basic cellular lesion in CNS of suckling rats treated with Pexid was studied by light and electron miroscopy. The most pronounced abnormality, the formation of various intracytoplasmic inclusions, was found in neurons, astrocytes, oligodendrocytes, ependymal cells, endothelial cells and fibroblasts. These abnormal inclusions were generally membrane-bound, although clearly non-membrane-bound inclusions were occasionally found. The several internal patterns of the inclusions were (1) lamellar, both concentric and parallel, (2) reticular and (3) crystalloid. These alterations were completely reversed following withdrawal of the drug.

The structural characteristics of the abnormal inclusions in Pexid-treated animals were similar to those found with certain hypocholesterolemic, neuroleptic, anorectic, and antimalarial drugs. This suggests that the inclusions occurring within the cells of animals treated with any of these drugs may develop in a similar manner, and that the formation of such inclusions is likely to be a form of cellular reaction common to certain metabolic disturbances.

Key words: Perhexiline maleate – Pexid – Abnormal inclusion – Rat – CNS.

Pexid[1] is an agent that has been studied to determine its effectiveness in the relief of angina pectoris (Winsor, 1970; Lyon, 1971). It produces a transient vasodilation of the coronary vascular bed, the pharmacological mechanism of action of which is not clearly understood. The effect of Pexid on the coronary vascular bed is not mediated by alpha or beta receptor stimulation, nor does it inhibit adenosine deaminase.

Clinical symptoms referable to the peripheral nerves or skeletal muscles have been reported in patients who have received Pexid. Biopsies of peripheral nerve, skeletal muscle and skin from these patients have shown the presence of membrane-bound, lamellated or crystalloid, intracytoplasmic inclusions (Bousser et al., 1975; Lhermitte et al., 1976; Mussini et al., 1977).

Similar lipidosis-like cellular alterations have been induced by a variety of drugs, such as AY9944 (Suzuki et al., 1973), triparanol (Yates et al., 1967; Chen and Yates, 1967; Schutta and Neville, 1968; Suzuki et al., 1974), chlorophentermine (Lüllmann-Rauch, R., 1974; Anzil et al., 1974), chloroquine (Fedorko, 1968; Hendy et al., 1969; Abraham et al., 1968; Abraham and Hendy, 1970; Read and Bay, 1971), and others.

The present study was designed to determine whether Pexid would also induce abnormal inclusions in the central nervous system (CNS) and other major body organs of rats. This paper concerns observations made in the CNS; the effects upon other organ systems will be dealt with in future communications (Jung and Suzuki, in preparation).

Materials and Methods

Twenty-six animals, including six controls, were studied. Twelve-day-old Charles River CD rats of the Sprague-Dawley strain were given Pexid, 300 mg/kg/day orally, using a 2% suspension of gum arabic. Control rats received a corresponding volume of 2% gum arabic only. Five doses were given weekly to each rat and delivered via a gastric cannula. The first group (I), consisting of four Pexid-treated and two control rats, was sacrificed at the age of 19 days after receiving five doses. The second group (II), consisting of four Pexid-treated and two controls rats, was sacrificed at the age of 26 days after receiving ten doses. The drug was discontinued for the third group (III), consisting of two Pexid-treated and two control rats, at the age of 26 days, and they were sacrificed 2 weeks later. After anesthetization with intraperitoneal Nembutal, the animals were perfused

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[1] Pexid is the trademark for perhexiline maleate, synthesized by Merrell-National Laboratories, Cincinnati, Ohio, 45215, U.S.A. Its chemical formula is 2-(2,2'-dicyclohexylethyl)-piperidine maleate

0001-6322/78/0042/0159/$ 1.20

intracardially with 2.5% glutaraldehyde in phosphate buffer at pH 7.4. Small blocks of tissue were taken from cerebrum, cerebellum, and spinal cord, post-fixed in Dalton's solution, dehydrated through graded ethanol, cleared in propylene oxide and embedded in Epon 812. They were sectioned with an LKB ultramicrotome at 1 ~ 2 μ and stained with toluidine blue for light microscopy. Representative areas were selected from these sections for electron microscopy. Thin sections were stained with uranyl acetate and lead citrate, and examined with a Phillips 200 electron microscope.

Results

Clinical Observation

The average weight of the animals used in this study was 30 g at the age of 12 days. At 19 days of age, a significant difference in weight was noted, the average weight being 45 g for the control and 25 g for the Pexid-treated animals. By that time, seven of the Pexid-treated animals had died and the rest exhibited a variety of signs including decreased locomotor activity, coarse fur, moderately flexed body posture, weakness of hindlimbs, wobbling gait, and fine generalized tremor. Some of the Pexid-treated animals experienced generalized seizures accompanied by screeching. By the age of 26 days, after receiving ten doses, the neurologic signs were less pronounced. At that time, the control animals weighed on the average 60 g and the Pexid-treated animals 30 g. Only three Pexid-treated animals died between days 19 and 26. After discontinuation of the drug at 26 days of age, the neurologic deficits gradually disappeared over a period of about 2 weeks.

Light Microscopy

There was no significant difference between Groups I and II at the light microscopic level. All of the Pexid-treated animals in these groups had cells with osmiophilic cytoplasmic inclusions throughout the CNS. Virtually all types of cells – neurons, astrocytes, oligodendrocytes, ependymal cells, endothelial cells and fibroblast – were affected. Pyramidal neurons in the entorhinal cortex (Fig. 1), cerebellar Purkinje cells (Fig. 2) and anterior horn cells of the spinal cord exhibited the most pronounced alterations. In these three types of cells there were, on the average, about 30 inclusions per cell perikaryon. The small nerve cells in the granular cell layer of the cerebellum contained fewer and smaller inclusions than neurons elsewhere in the CNS. Occasional glial cells were packed with extremely large, irregular inclusions. In the white matter, occasional axons and oligodendrocytes contained osmiophilic inclusions, but myelin sheaths and axons were otherwise of normal appearance.

The cells in animals from Group III and in control animals were of similar appearance; they contained only a few small osmiophilic dense bodies.

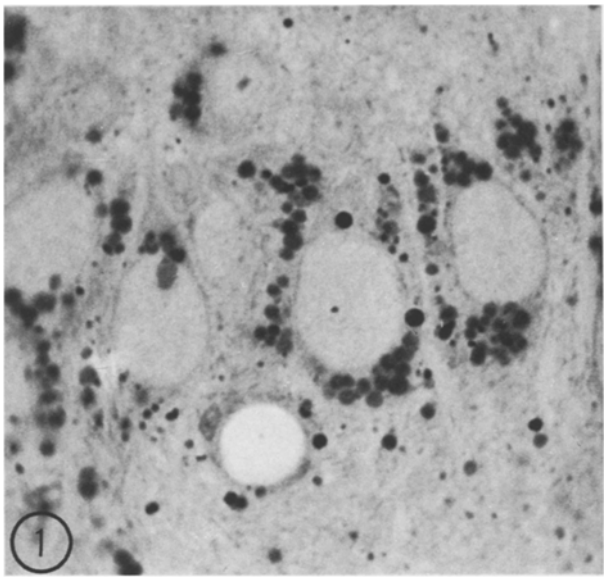

Fig. 1. Pyramidal neurons in the entorhinal cortex contain numerous osmiophilic inclusions of various size (Toluidine blue stain). Pexid-treated rat (Gr. I) ×1400

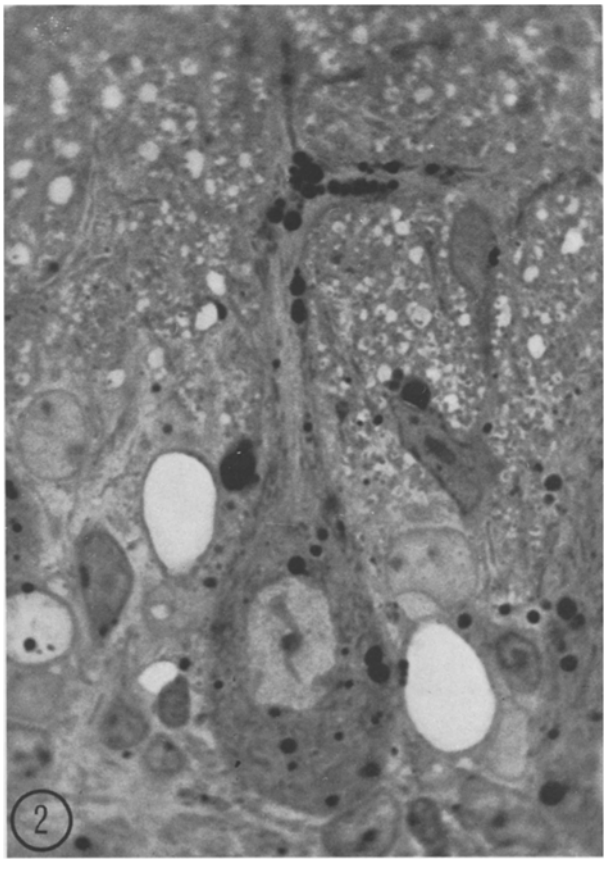

Fig. 2. Many osmiophilic inclusions are seen in the perikaryon and dendrite of the cerebellar Purkinje cell, and in the adjacent glial cells (Toluidine blue stain). Pexid-treated rat (Gr. I) ×1400

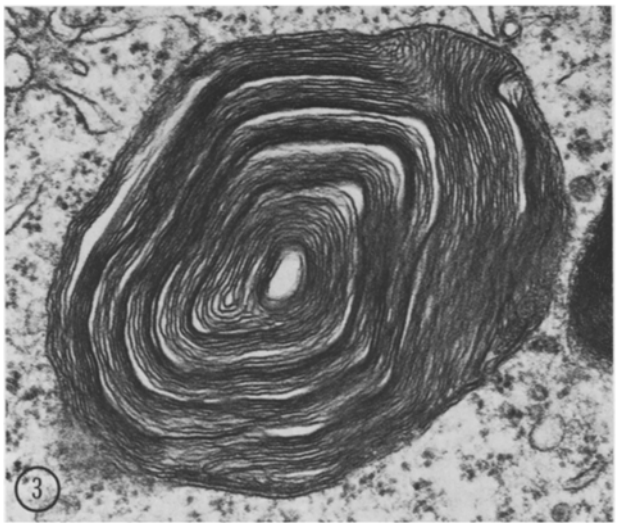

Fig. 3. A lamellar inclusion in the cerebellar Purkinje cells is composed of loosely arranged concentric and partly parallel membranes. Pexid-treated rat (Gr. I) ×42910

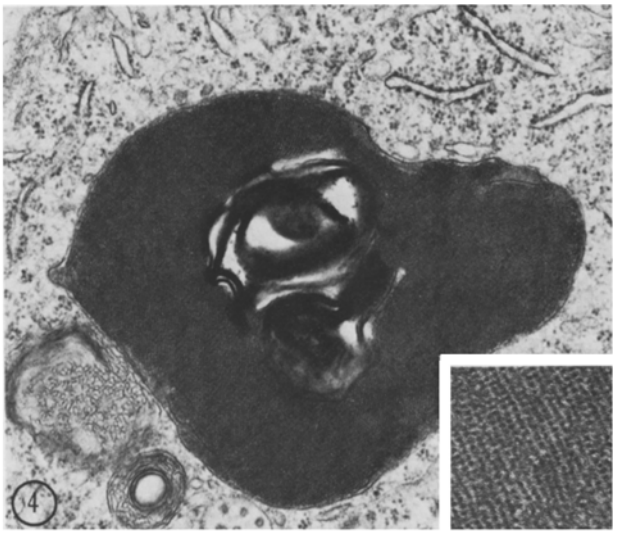

Fig. 4. An inclusion in the cerebellar Purkinje cells shows mostly crystalloid internal pattern with a focally compact lamellated structure in the center. Two small reticular inclusions are also seen at the left lower corner. Pexid-treated rat (Gr. I) ×36000. The inset revealed lattice-like crystalloid appearance. ×81455

Electron Microscopy

The osmiophilic granules seen by light microscopy in neurons from control and Group III animals proved to be either intracytoplasmic dense bodies or lipofuscin granules. They contained a finely granular matrix of moderate electron density. On rare occasion, a few short linear electron-dense structures were visible.

Findings in the Pexid-treated animals of Group I and II were similar. Ultrastructurally, the intracytoplasmic osmiophilic inclusions were seen to be of concentric and/or parallel lamellar (Fig. 3), crystalloid (Fig. 4), or reticular (Fig. 5) configuration. These internal patterns were often seen in combination (Fig. 4). The inclusions were largely bound by a limiting membrane. In the larger inclusions, however, a surrounding membrane was not always identified. The diameter of the inclusions varied between 0.5 and 4.0 μ.

Lamellar inclusions showed a variable degree of compactness. In compact areas, the electron-dense lamellae displayed a regular periodicity. In some areas the electron-dense lamellae were widely separated by electron-lucent spaces (Fig. 3). Occasionally, inclusions were found consisting partly of an amorphous dense matrix, from which the lamellae appeared to emerge. Rarely, there was a suggestive feature that a few of the lamellae were continuous with the limiting membrane of the inclusion. The inclusions composed of compact lamellae appeared to be electron dense irregular bodies at lower magnification (Fig. 5). At high resolution, however, these compact lamellae displayed an average periodicity of about 45 Å. Within oligodendrocytes lamellar inclusions often appeared to have fused together to occupy large areas within the cytoplasm (Fig. 6).

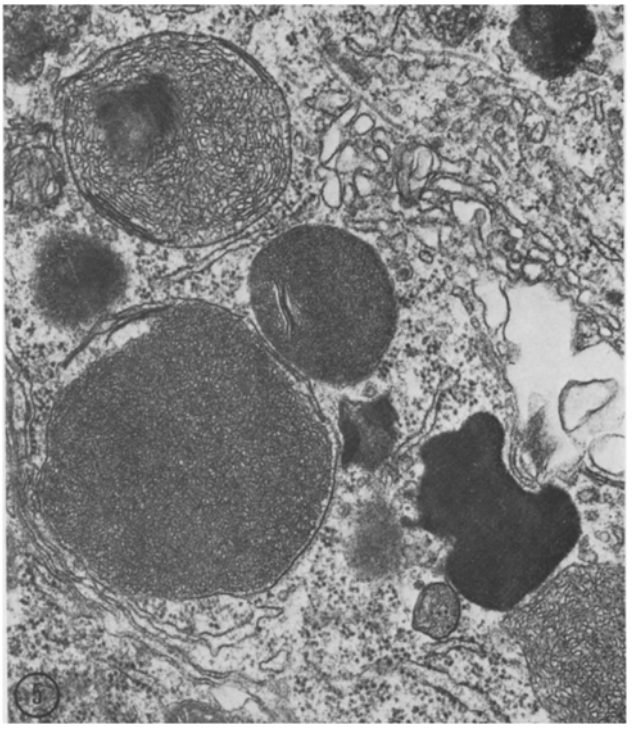

Fig. 5. Reticular inclusions and a compact electron-dense lamellar inclusion in the cerebral cortical neuron. Pexid-treated rat (Gr. II) ×27510

Reticular inclusions showed a disordered labyrinthine or entangled membranous appearance (Figs. 4, 5).

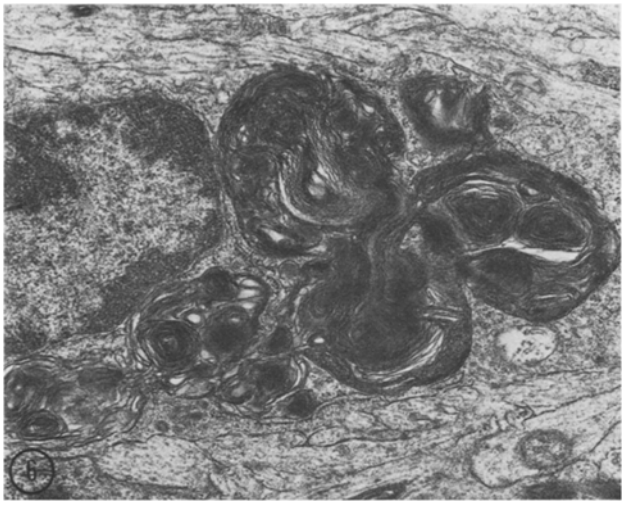

Fig. 6. Large conglomerated lamellar inclusions are observed within the oligodendrocyte in the cerebral white matter. Pexid-treated rat (Gr. II) × 7965

Their arrangement was often quite irregular; however, in some instances there appeared to be a rather uniform mosaic of polygonal profiles which at its borders seemed continuous with the limiting membrane.

Crystalloid inclusions were of orderly lattice-like configuration (Fig. 4). The plane of orientation of the crystalloid subunits often varied from one area to the next within the same inclusion. Occasionally, the lattices appeared to be of less compact and slightly less regular organization.

The most frequently encountered type of inclusion was the lamellar inclusion (Figs. 3, 5), which was identified in all types of cells throughout the CNS. Reticular inclusions (Figs. 4, 5) were found much less frequently, but showes no distributional localization. Crystalloid inclusions were seen almost exclusively with in Purkinje cells (Fig. 4), although rare astrocytes within the cerebrum also contained this type of inclusion.

Certain other less specific ultrastructural alterations were identified which were more prominent in experimental than in control animals. Lysosomes and autophagic vacuoles were abundant within cerebral neurons, Purkinje cells and anterior horn cells of the spinal cord. In some instances, it was somewhat difficult to distinguish them from the abnormal inclusions, which assumed a multitude of shapes and configurations. In some areas of cytoplasm free from the abnormal inclusions, autophagic vacuoles could be detected. The Golgi complex and endoplasmic reticulum appeared to be more prominent. The Golgi vesicles appeared more numerous than those of the controls. Abnormal inclusions were frequently found in the vicinity of the Golgi complex. Multivesicular bodies were often found nearby.

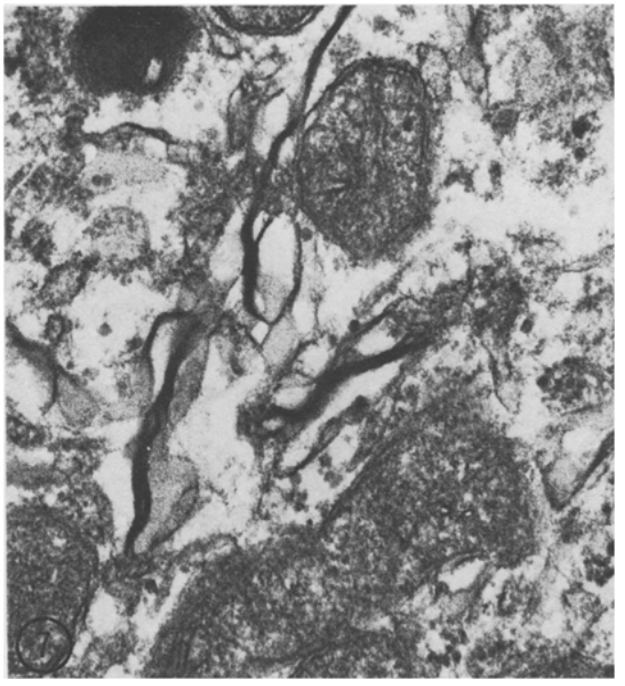

Fig. 7. A loose aggregate of irregular membranous structures is seen within the cerebellar Purkinje cell. They appear to have no connections with any of the pre-existing structures. Pexid-treated rat (Gr. II) 57850

Very infrequently, loose aggregates of irregular membranous structure were noted within the cytoplasm of Purkinje cells. We are not certain of the nature of these aggregates; they may be artefactitious, but it is also possible that they are structurally related to the abnormal inclusions (Fig. 7).

In the white matter, lamellar inclusions were not infrequently found in both myelinated and unmyelinated axons and in dendrites. In addition, degenerating axons containing numerous dense bodies, some of which were lamellated, were also encountered (Fig. 8). The myelin sheaths, however, were generally well preserved.

Discussion

The ultrastructural appearance of the inclusions within the CNS of our experimental animals is essentially identical to that observed in biopsies of peripheral nerve, skeletal muscle and skin from patients who were treated with Pexid (Bousser et al., 1976; Lhermitte et al., 1976; Mussini et al., 1977). This report constitutes the first experimental evidence that Pexid induces, within the CNS, the formation of abnormal intracytoplasmic inclusions. It also provides evidence of their morphologic similarity to those observed with the administration of certain anorectic, antimalarial, neuroleptic and hypocholesterolemic agents.

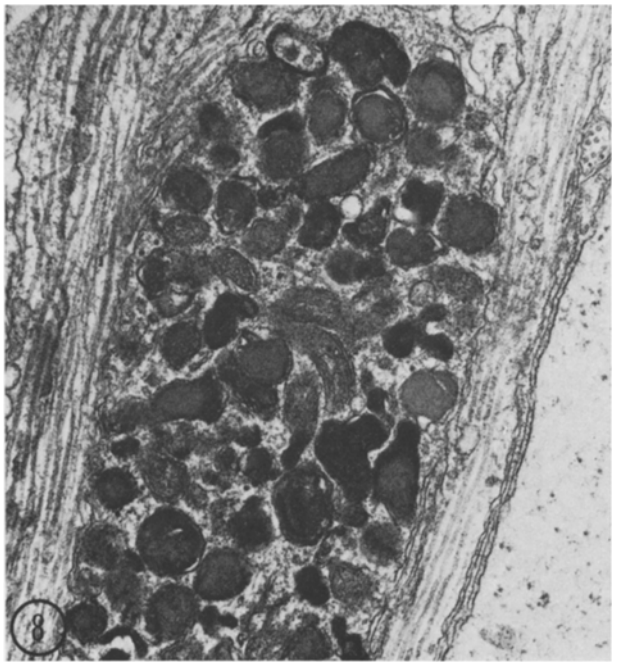

Fig. 8. A degenerating axon in the cerebral white matter contains numerous intra-axonal dense and/or lamellar bodies and mitochondria. Pexid-treated rat (Gr. II) ×21695

Administration of triparanol, a tricyclic antidepressant and hypocholesterolemic agent, for example, results in the production of strikingly similar changes within the CNS. Chen and Yates (1967) classified the inclusions induced by triparanol into four types: membranous whorls (Type I), labyrinthine aggregates of smooth membranes (Type II), dense bodies with a reticular internal structure (Type III), and crystalloid bodies showing a regular lattice pattern (Type IV). The inclusions induced by Pexid in our experimental animals exhibited the entire spectrum of changes from Type I to Type IV.

Formation of one or another type of inclusion or inclusions of mixed internal pattern may depend on the accumulation of lipid within a given cell type, and on certain other physicochemical parameters of the intracellular environment (Lüllman-Rauch, 1974). Transitional forms between lamellar and crystalloid arrays can be induced by alterations of pH, Ca^{++} concentration and hydration, or by the presence of certain amino acids and fusogenic lipids (Stoeckenius, 1962; Junger and Reinauer, 1969; Kreutz, 1972; Howell et al., 1973). Such environmental changes might therefore account for the occurrence of inclusion bodies of variable pattern in this study.

Lamellar inclusions of similar size and appearance have been described within the neurons of patients with gangliosidoses (Terry and Weiss, 1963), in which the isolated lamellar inclusions contained significant amounts of ganglioside (Suzuki and Suzuki, 1972). Lamellar and crystalloid inclusions were reported in mucolipidosis IV, in which the ganglioside level was also increased (Tellez-Nagel et al., 1976). Recently, an increase in ganglioside content of peripheral nerve (Pollet et al., 1977) and storage of abnormal ganglioside in the liver (Lageron et al., 1977) have been reported in patients receiving Pexid.

Histochemically and biochemically, Yates (1966), Arai et al. (1967), and Yates et al.(1967), in experiments with triparanol, demonstrated a high phospholipid content within the crystalloid inclusions. Their lattice-like internal structure somewhat resembles the hexagonal patterns adopted by some phospholipids under certain in vitro conditions (Stoeckenius, 1962). To our knowledge, however, no experimental study has yet been made in which CNS inclusions of the type we are discussing have been isolated or characterized biochemically.

Despite the morphological similarity between the changes induced by Pexid and those induced by other drugs, the structural formulas of the latter differ considerably from that of Pexid. Abnormal inclusion formation has been repeatedly ascribed to the amphophilic character of many of these agents. Presumably, these compounds react with polar lipids such as phospholipid, a mayor constituent of membranous structures within cells. Strictly from a physicochemical point of view, the nitrogen atom in the heterocyclic ring of Pexid would likely carry a cationic charge in the aqueous state, whereas the dicyclohexylethyl group appears inactive in terms of polarity. Pexid, if amphophilic, is probably only weakly so. Drenckhahn et al. (1976) have suggested that more weakly amphophilic drugs require larger dosages for the induction of abnormal inclusions.

Since the appearance of similar inclusions may result following the administration of a variety of agents, their formation is not likely to be due to a specific cytotoxic effect, as Schutta and Neville (1968) postulated in their experiment with cholesterol synthesis inhibitors. The pathogenesis of these inclusions is perhaps similar with all of these drugs. Regardless of the pathogenesis, at least some of the effects of these agents are reversible, as evidenced by the disappearance of the abnormal inclusions once the agent is discontinued. In view of the presence of autophagic vacuoles and multivesicular bodies, interference with certain lipid metabolism by inclusion-inducing drugs has been thought to be involved in the formation of the inclusions (Hruban et al., 1972; Fedorko et al., 1968a and b). The present findings are consonant with this suggestion. The actual origin of the inclusions, however, their significance for cell function, and the bio-

chemical nature of the disturbance require further investigation.

In conclusion, the present study provides morphological evidence that Pexid can induce the formation of abnormal inclusions within the CNS of the suckling rats. It also suggests that Pexid may possibly induce a cellular disturbance similar to that associated with the administration of certain hypocholesterolemic, anoretic, neuroleptic or antimalarial drugs, on the basis of the close resemblance of the ultrastructural alterations caused by these agents. The ultimate common denominator, if any, for the genesis of these abnormalities is yet to be determined.

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References

Abraham, R., Hendy, R., Grasso, P.: Formation of myeloid bodies in rat liver lysosomes after chloroquine administration. Exp. Molec. Path. **9**, 212–228 (1968)

Abraham, R., Hendy, R.: Irreversible lysosomal damage induced by chloroquine in the retinae of pigmented and albino rats. Exp. Molec. Path. **12**, 185–200 (1970)

Arai, K., Yates, R. D., Rappoport, D.A.: Fine structure and chemical composition of opaque cytoplasmic bodies of triparanol treated Syrian hamsters II. Phospholipid analysis of opaque bodies from adrenal glands. Tex. Rep Biol. Med. **25**, 350–359 (1967)

Anzil, A. P., Herrlinger, H., Blinzinger, K.: Lipidose des Nervensystems nach Chlorphentermin-Applikation. Die Naturwissenschaften **61**, 35–36 (1974)

Bousser, M. G., Bouche, P., Brochard, C., Herreman, G.: Neuropathies périphériques au myléate de perhexiline. A propos de 7 observations. Coeur Méd., Int. **15**, 181–188 (1976)

Chen, I. L., Yates, R. D.: An ultrastructural study of opaque cytoplasmic inclusions induced by Triparanol treatment. Am. J. Anat. **121**, 705–725 (1967)

Drenkhahn, D., Kleine, L., Lüllman-Rauch, R.: Lysosomal alteration in cultured macrophages exposed to anorexigenic and psychotropic drugs. Lab. Invest. **35**, 115–123 (1976)

Fedorko, M. D., Hirsch, J. G., Cohn, Z. A.: Autophagic vacuoles produced in vitro. I. Studies on cultured macrophages exposed to chloroquine. J. Cell Biol. **38**, 377–391 (1968a)

Fedorko, M. D., Hirsch, J. G., Cohn, Z. A.: Autophagic vacuoles produced in vitro. II. Studies on the mechanism of formation of autophagic vacuoles produced by chloroquine. J. Cell Biol. **38**, 392–402 (1968b)

Fedorko, M. D.: Effect of chloroquine on morphology of leukocytes and pancreatic exocrine cells from the rat. Lab. Invest. **18**, 27–37 (1968)

Hendy, R. J., Abraham, R., Grasso, P.: The effect of chloroquine on rat heart lysosomes. J. Ultrastruct. Res. **29**, 485–495 (1969)

Howell, J. I., Fisher, D., Goodall, A. H., Verrinder, M., Lucy, J. A.: Interactions of membrane phospholipids with fusogenic lipids. Biochem. Biophys. Acta (Amst.) **332**, 1–10 (1973)

Hruban, Z., Slesers, A., Hopkins, E.: Drug-induced and naturally occurring myeloid bodies. Lab. Invest. **27**, 62–70 (1972)

Jung, H. J., Suzuki, K: Morphological changes in body organs of rats treated with Perhexiline maleate (Pexid). (in preparation)

Junger, E., Reinauer, H.: Liquid crystalline phases of hydrated phosphatidyl ethanolamine. Biochem. Biophys. Actat (Amst.) **183**, 304–308 (1969)

Kreutz, W.: Strukturprinzipien in Bio-membraner. Angew. Chem. **84**, 597–614 (1972)

Lageron, A., Poupon, R., DeSaint-Maur, P. P., Levy, V. G.: Liver anglioside storage after perhexiline maleate. Lancet **I**, 483 (1977)

Lhermitte, F., Fardeau, M., Chedu, F., Mallecourt, J.: Polyneuropathy after perhexiline maleate therapy. Brit. Med. J. **1**, 1256 (1976)

Lüllmann-Rauch, R.: Lipidosis-like alterations in spinal cord and cerebellar cortex of rats treated with chlorphentermine or tricylic anti-depressant. Acta neuropath. (Berl.) **29**, 237–249 (1974)

Lyon, L. J., Nevins, M. A., Fisch, S., Henry, S.: Perhexiline maleate in treatment of angina pectoris. Lancet **I**, 1272–1274 (1971)

Mussini, J. M., Hauw, J. J., Escourolle, R.: Etude en microscopie électronique des lésions nerveuses, musculaires et cutanées déterminées par le maléate de perhexiline. Acta neuropath. (Berl.) **38**, 53–59 (1977)

Pollet, S., Hauw, J. J., Escourolle, R., Baumann, N.: Peripheral nerve lipid abnormalities in patients on Perhexiline maleate. Lancet **I**, 1258 (1977)

Read, W. K., Bay, W. W.: basic cellular lesion in chloroquine toxicity. Lab. Invest. **24**, 246–259 (1971)

Schutta, H. S., Neville, H. E.: Effects of cholesterol synthesis inhibitors on the nervous system. Lab. Invest. **19**, 487–493 (1968)

Stoeckenius, W.: Some electron microscopical observations on liquid crystalline phase in liquid-water systems. J. Cell Biol. **12**, 221–229 (1962)

Suzuki, K., Zagoren, J. C., Gonatas, J., Suzuki, K.: Ultrastructural study of neuronal cytoplasmic inclusions produced by hypocholesterolemic drug AY9944. Acta neuropath. (Berl.) **26**, 185–197 (1973)

Suzuki, K., Suzuki, K.: Pathological cytosomes. In: Handbook of Neurochemistry. Vol. 7, pp. 131–142. New York: Plenum Press (1972)

Suzuki, K., Zagoren, J. C., Chen, S. M., Suzuki, K.: Effect of triparanol and 20,25-diazacholesterol in CNS of rat; Morphological and biochemical studies. Acta neuropath. (Berl.) **29**, 141–150 (1974)

Tellez-Nagel, I., Rapin, J., Iwamoto, T., Johnson, A. B., Norton, W. T., Nitowski, H.: Mucolipidosis IV. Arch. Neurol. **33**, 828–835 (1976)

Terry, R. D., Weiss, M.: Studies in Tay-Sachs disease. II. Ultrastructure of cerebrum. J. Neuropath. Exp. Neurol. **22**, 18–55 (1963)

Winsor, T.: Clinical evaluation of perhexiline maleate. Clin. pharmacol. Ther. **11**, 85–89 (1970)

Yates, R. D.: The effects of triparanol on adrenocortical cells of the zona fasciculata of Syrian hamsters. Z. Zellforsch. **71**, 41–52 (1966)

Yates, R. D., Arai, K., Rappoport, D. A.: Fine structure and chemical composition of opaque cytoplasmic bodies of triparanol treated Syrian hamsters. Exp. Cell Res. **47**, 459–478 (1967)

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