CRYSTALLINE INCLUSIONS IN THE
NUCLEAR ENVELOPE AND GRANULAR
ENDOPLASMIC RETICULUM OF THE
FISH SPINAL CORD

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ABSTRACT

Crystalline inclusions were found in the nuclear envelope and granular endoplasmic reticulum of spinal cord oligodendroglia of the common guppy (Poecilia reticulata) and the lungfish (Polypterus enlicheri). A considerably increased incidence of these inclusions was noted in guppies with congenital and hereditary (sex-linked, recessive) lordosis. Identical crystalline inclusions were observed in protoplasmic and fibrous astrocytes, ependymal cells, and capillary endothelial cells of the spinal cord of the lordotic fish. The oligodendroglia in these fish also revealed a prominent alteration of the endoplasmic reticulum and Golgi apparatus, with the accumulation of dense (secretion?) granules and large amounts of electron-opaque material in markedly dilated sacs of the endoplasmic reticulum. The authors postulate that this alteration is caused by a genetic defect in the control mechanism governing the elaboration of this material in the lordotic guppy, with subsequent stasis and crystallization of this material.

INTRODUCTION

Electron microscopic investigations have revealed intracellular crystalline inclusions in a wide range of organisms: plants (3, 7, 27), protozoans (15), coelenterates (9), echinoderms (21), annelids (23, 29), arthropods (2, 35), amphibians (18, 19, 20, 42), fish, birds (41), and mammals (4-6, 10-13, 16, 17, 22, 24, 26, 32-34, 36-38). Various crystalline inclusions have been found free in the cytoplasmic matrix (13, 15, 19, 22, 24, 32, 33), in plastids (3, 27), mitochondria (9, 20, 34, 36, 42), secretory granules (12), microbodies (10), endoplasmic reticulum (4-7, 11, 16, 18, 26, 38, 41, and footnote 1), Golgi apparatus (17, 23, 35), nucleus (16, 21, 34, 39, 40, and specialized crystallogenic bodies (37). The occurrence of crystalline inclusions in nonneoplastic lesions of the central nervous system has not been reported before. Twice previously, dissimilar crystalline cytoplasmic inclusions were noted in the astrocytic tumors in man (22, 24).

In the present study crystalline inclusions were observed in the nuclear envelope and granular endoplasmic reticulum of oligodendroglia of two widely separated species of fish representing both ends of the phylogenetic line of the subclass Actinopterygii. These two species are the common guppy (Poecilia reticulata) belonging to the suborder Teleostei and the primitive lungfish (Polypterus enlicheri) belonging to the suborder Chondrostei (considered to be a direct descendant of the...
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Crystalline Inclusions in Nuclear Envelope

MATERIAL AND METHODS

Adult lungfish (Polypterus enlicheri) and adult normal guppies (Poecilia reticulata) obtained from the Aquarium Stock Co. Inc., New York, and normal and lordotic guppies bred by the senior author, were kept in a conventional aquarium at 78°F and pH 7.0. The lordotic condition appeared as a result of successive inbreedings of delta tail guppies over a period of 2 years. Female guppies were used exclusively since the pigmentation of the male obscures the visualization of the spinal cord, and the lordotic condition noted in this study appeared only in females.

The fish were anesthetized with tricaine methane-sulfonate (Sandoz, Inc., New York), and 1 mm pieces of spinal cord were removed from an area below the dorsal fin. A few tissue blocks were fixed in 4% cacodylate-buffered glutaraldehyde (31) for 1 hr, washed in buffer overnight, minced with a sharp razor blade, incubated in Gomori's acid phosphatase medium (14), and postosmicated. Most tissue blocks were immersed directly in 2% chrome-osmium fixative (8) for 2 hr at 4°C. All tissue blocks were then dehydrated with graded alcohols and propylene oxide at room temperature and embedded in Araldite 502. Thin sections were cut with diamond knives and mounted on bare copper grids. The sections were stained with a saturated solution of uranyl acetate in ethyl alcohol followed by lead hydroxide (25), carbon coated for stability, and examined with Hitachi HS7 (Hitachi, Ltd., Tokyo, Japan) and RCA EMU 3F electron microscopes.

RESULTS

Although the criteria for the differentiation of glial cells is not as clear-cut in fish as in mammals, it is possible to distinguish oligodendroglia, and fibrous and protoplasmic astrocytes. The processes of the astrocytes are found encircling capillaries. These cells have few organelles and a relatively simple internal structure (Figs. 11 and 12). The oligodendroglia have a more complex cytoplasmic structure, with a well developed endoplasmic reticulum, denser cytoplasm, and many mitochondria. This cell is often found enveloping and apparently myelinating axons (Figs. 1 and 5). In both the normal lungfish and guppy, rare crystalline structures are noted in the granular endoplasmic reticulum and the nuclear envelope of oligodendroglia (Figs. 1, 2, 8, and 9) near the surface of the spinal cord (Fig. 1). It is estimated that only one such crystalline inclusion is noted after an exhaustive search of approximately 30 grids. These structures are variable in size, often cubic with angular profiles, and appear homogeneously granular (Figs. 2 and 9). Infrequently, these crystalline inclusions display a periodicity of 35 to 50 Å (Fig. 7). Occasionally, they appear to pinch off from the nuclear envelope, but remain enclosed within the rough endoplasmic reticulum.

In the normal fish the endoplasmic reticulum is moderately prominent and slightly dilated (Fig. 1). In inbred delta tail guppies, congenitally lordotic female fish (apparently due to a sex chromosome-linked recessive gene) were found to have many more crystalline inclusions. These inclusions were of similar size, shape, and internal structure, and were observed in almost every grid examined (Figs. 3–7). Identical crystalline structures were also frequently noted in these fish in many other cell types, including protoplasmic astrocytes (Fig. 11), fibrous astrocytes, ependymal cells (Fig. 10), and capillary endothelial cells (Fig. 12). These structures were observed within the granular endoplasmic reticulum and nuclear envelope as in the normal fish, and frequently were noted in what apparently are buds of the nuclear envelope.

The endoplasmic reticulum of the oligodendroglia of the lordotic fish is often strikingly dilated and contains granular electron-opaque material resembling that seen in actively secreting cells (e.g. plasma cells, thyroid follicular cells) (Fig. 3). The Golgi apparatus in these cells is similarly prominent with frequent budding. In the Golgi zone many oval, electron-opaque structures of variable size are noted. The smallest of these resemble Golgi vesicles and contain granular material of similar density. Many progressively larger and denser structures are present in this zone but they are somewhat more distant from the Golgi lamellae, suggesting a progressive accumulation and concentration of secretory material (Figs. 3 and 4). The material in the endoplasmic reticulum is less dense than that in these Golgi zone structures. Occasionally, osmiophilic material and myelin figures are noted in these cells (Fig. 4). Although abnormal oligodendroglia were noted frequently in lordotic guppies, no abnormality in myelinization of axons was appreciated.

Acid phosphatase preparations revealed activity in large, dense particles resembling “dense bodies.” These particles differed from the secretion granules...
FIGURE 1  Periphery of the spinal cord of the normal guppy, with the pia (P) at the edge of the illustration. Note a cubic crystalline inclusion (arrow) in a dilated sac of rough endoplasmic reticulum (which appears to bud from the nuclear envelope) of an oligodendroglial cell, processes of which envelop myelinated axons. There is slight dilatation of the endoplasmic reticulum of this cell. × 13,800.

FIGURE 2  The same inclusion noted in Fig. 1 at higher magnification, showing a finely granular internal structure. × 156,500.
FIGURE 3  An oligodendroglial cell of a lordotic guppy with a strikingly dilated endoplasmic reticulum containing granular electron-opaque material resembling that seen in actively secreting cells. The Golgi apparatus is prominent with frequent budding. In the Golgi zone, electron-opaque structures of variable size resembling Golgi vesicles and secretion granules are noted. These structures contain material more dense than that in the endoplasmic reticulum. $\times$ 26,000.
FIGURE 4  An oligodendroglial cell of a lordotic guppy with a cubic crystalline inclusion in a dilated sac of rough endoplasmic reticulum. Note that many of the oval granules containing electron-opaque material are similar to some of those (secretion granules?) seen in Fig. 3. Many similar granules (some at arrows) contain vesicles and myelin figures and resemble "dense bodies." Lipid droplets and a myelin figure appear to be in an autophagic vacuole. × 28,500.

FIGURE 5  An oligodendroglial cell of a lordotic guppy with a cubic crystalline inclusion in a dilated sac of rough endoplasmic reticulum. This cell appears to be myelinating an axon (arrow). × 32,000.
Figure 6. An oligodendroglial cell of a lordotic guppy with a cubic crystalline inclusion in its nuclear envelope. × 59,000.

Figure 7. An oligodendroglial cell of a lordotic guppy with two crystalline inclusions, the largest of which exhibits a periodicity of 50 Å. × 92,000.
**FIGURE 8** An oligodendroglial cell of the lungfish with a cubic crystalline inclusion in the nuclear envelope (arrow). $\times 34,000$.

**FIGURE 9** The same inclusion noted in Fig. 8 at higher magnification showing its finely granular internal structure. $\times 136,600$. 
FIGURE 10  Ependymal cells of a lordotic guppy, with the central canal at the edge of the illustration at C. Note crystalline inclusions in dilated sacs of rough endoplasmic reticulum in two cells (arrows). X 12,900.

FIGURE 11  Note a cubic crystalline inclusion in a dilated sac of rough endoplasmic reticulum (arrow), in a protoplasmic astrocyte of a lordotic guppy. Capillary (C). X 11,100.

FIGURE 12  Note a cubic crystalline inclusion in a dilated sac of rough endoplasmic reticulum (arrow), in a capillary endothelial cell of a lordotic guppy. Capillary lumen (C). X 36,000.
FIGURE 13 Acid phosphatase preparation of oligodendrogial cell of a lordotic guppy. Note unstained crystalline inclusion and dense (secretion?) granules. Many similar granules contain acid phosphatase activity. Some of these contain vesicles (arrow) and resemble "dense bodies". X 48,700.

insofar as they contained vesicles, myelin figures, and other structures commonly noted in lysosomes (Figs. 4 and 13). No acid phosphatase activity was observed in the endoplasmic reticulum, nuclear envelope, secretion granules, crystalline structures, the Golgi apparatus, or in the Golgi zone vesicles (Fig. 13).

DISCUSSION
The present report indicates that crystalline structures that are infrequently observed in the endoplasmic reticulum and nuclear envelope of oligodendroglia of normal guppy and lungfish spinal cord are seen in increased number in this cell and in various other cell types in which they were not previously noted (e.g. fibrous and protoplasmic astrocytes, ependymal cells, and capillary endothelial cells) in the spinal cord of lordotic guppies. Accompanying this finding in the oligodendroglia in lordotic guppies is a striking dilatation of the endoplasmic reticulum, which is seen to contain an abundance of granular electron-opaque material, as well as an increase in the size, complexity, and budding of the Golgi apparatus with an accumulation of dense (secretion?) granules. It is postulated that the volume of protein produced exceeds the volume which the Golgi apparatus can package and which the cell can utilize, and that subsequent accumulation and crystallization of this material occur in the nuclear envelope and dilated sacs of the endoplasmic reticulum.

In lordotic fish, those oligodendroglia with distended sacs of endoplasmic reticulum and a
large, apparently active Golgi zone resemble actively secreting cells (e.g. plasma cells, thyroid follicular cells). Similar cells, resembling secretory cells, have previously been observed in the central nervous system but only in a few specialized neurosecretory systems (1, 28). Crystalline inclusions have not been found previously in the central nervous system, except on rare occasions as ill-defined cytoplasmic inclusions in astrocytic nervous system, except on rare occasions as ill-defined cytoplasmic inclusions in astrocytic tumors (22, 24). Crystalline inclusions occur in astrocytes, ependymal cells, and capillary endothelial cells in lordotic guppies only, but their incidence is considerably lower in those cells than in oligodendroglia. Alterations of the endoplasmic reticulum and Golgi apparatus were not observed in these cells. Cells of other organs of the lordotic fish were not examined. 

Crystals not unlike those noted in this work were observed in the endoplasmic reticulum of plasma cells by Bessis (5) and Thiéry (38). These workers believed that the crystals were formed from high molecular weight proteinaceous substances elaborated by the endoplasmic reticulum and that they were trapped in this reticulum and subsequently crystallized. Oval granules, which resembled secretion granules, and homogeneously granular cubic crystals were observed by Lindler (23) in earthworm connective tissue cells. He thought that this material was produced in both the perinuclear cisternae and the endoplasmic reticulum and that it was concentrated in the Golgi apparatus. Petzold (29) studied these cubic crystals and noted that an occasional crystal showed a periodicity of 35 to 70 Å. Those structures resemble the crystalline inclusions noted in the present communication, except that they were found in sacs of agranular reticulum. Strunk (35) and Hadek and Swift (17) have also implicated the Golgi apparatus in crystal formation. Only Behnke and Moe (4) found similar crystals in the nuclear envelope. They also noted these crystals in the endoplasmic reticulum of Paneth cells of the rat.

It is generally agreed that these and similar crystals are proteinaceous (6, 16, 18, 23, 38-40). An increase in the number of crystalline structures has been noted in glandular stomachs of fasted animals (41), indicating that the crystals may be storage products in the resting cells. Hamilton et al. (18) have related crystals found in the slender salamander to reserve product utilized during estivation. Fawcett and Burgos (13) have indicated that similar crystals might represent storage forms related to normal metabolic activities of the cell.

An increase in the numbers of crystals has also been observed in regenerating animals (9) where active protein secretion is needed for tissue repair and rebuilding, and crystals have been found in the endoplasmic reticulum of beta cells in the fish pituitary and of fish melanoma cells (results of which are to be published). In contradistinction, Tandler and Shipkey (37) consider that the crystals in Warthin's tumor are representative of trapped secretory product.

In the present report the authors assume that the uncrystallized material present in the dilated sacs of rough endoplasmic reticulum and in the round, dense granules (secretion granules?) is identical in composition with, although less concentrated than, the cubic crystals found in the nuclear envelope and endoplasmic reticulum. Hamilton et al. (18) noted that serum lipoprotein may form similar cubic crystals in tissue. Although the crystalline structures illustrated in the present work show a periodic structure different than that noted by Hamilton et al. (18), which probably indicates a different composition, this difference in periodic structure might also be accounted for by differences in processing (33). Visualization of crystalline structure is dependent on fixation and sectioning as well as on the plane of section and, in general, on the embedding procedure. The endoplasmic reticulum is involved in lipoprotein production. It seems probable that in the oligodendroglia in the normal animal this protein is held in reserve to be used in the process of myelinization. The authors think that in lordotic fish the general genetic control mechanism governing the elaboration of this protein is defective and that this defect causes an abnormal accumulation of protein in many cell types. Since myelinization appears normal and since similar crystals are noted in both the lordotic and normal fish, the accumulation of this protein material in lordotic fish probably does not indicate an inability to utilize this material but rather indicates an overproduction of it, although either one or a combination of these factors is a possible explanation. It is also possible that different proteins are responsible for crystals in the various cell types. Tissues other than the spinal cord have not been investigated to determine whether this accumulation is also present in other organs. No periodicity was noted in these crystals in the lungfish, probably indicating a difference in their chemical composition which could be expected in such widely divergent species.
even if the crystals in both species represented lipoproteins.

Lordosis in guppies has been investigated by Rosenthal and Rosenthal (30) who found the lesion in fish of both sexes and considered it an autosomal, single gene, recessive mutation. In a second lordotic strain, these investigators were not able to determine a genetic link. In the present study only female guppies become lordotic, indicating that the defect is probably sex-linked in its inheritance.

REFERENCES


