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Unusual circular annulate lamellae in hepatocytes of *Torpedo marmorata*

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Summary. This report describes an unusual morphology of annulate lamellae (AL) in the hepatocytes of *Torpedo marmorata* Risso. These Als and fragments are detected amidst the main glycogen and lipid deposits. AL cisterns are circumscribed by parts of the smooth endoplasmic reticulum. Based on the finding of these unusual annular ALs, accompanied by other subcellular lesions such as a number of membranous whorls and altered mitochondria. These findings can concur and support other authors' observations suggesting that these adult hepatocytes transient changes reflect that this species could be exposed to local, natural or likely human coastal seabed pollutants.

Key words: Annulate lamellae, Liver, Microscopy, Hepatocyte, Torpedo marmorata, Cytotoxicity

Introduction

The term annulate lamellae (AL) can be used to describe an intracellular organelle composed of parallel arrays of cisterns bearing at regular intervals small annuli or fenestrrae. AL ressemble stacked pieces of nuclear envelope. In a few exceptional instances they have been detected in continuity with the nuclear envelope (Kessel, 1983) and have been located intranuclearly (Ghadially and Parry, 1974, surveys by Ghadially, 1985, 1997; Kessel 1983, 1992). AL contains ribonucleoproteins attached to their outer surface or to some of the endoplasmic-associated membranes. Originally observed by a few histologists in the oocytes of marine and terrestrial invertebrates, pancreatic acinar cells and rat spermatids, it is the electron microscope that allowed Swift (1956) to first coin the term. Several other terms have been used in describing this cell infrastructure: "coarse fibrous component", "periodic lamellae' (Rebhun, 1956), "secondary membranes" (Merriam, 1959), "pitted membranes" and "porous cytomembranes" (Kessel, 1968a). If ALs were reviewed by Witschnitzer (1970), Ghadiallay (1985, 1997), these organelles were the topic of numerous publications by Kessel (1968a,b, 1983, 1992). It can be noted that these cell substructures are usually located in the cytoplasm of vertebrate and invertebrate germ cells, embryonic cells and several animal benign and malignant tumor cells (Wischnitzer, 1970; Kessel, 1983, 1992; Ghadially, 1997). However this organelle is also found occasionally in some adult somatic cells. AL may also present complexes surrounded by adjacent membranous saccules of smooth or rough endoplasmic reticulum (ER). ER saccules are eventually continuous or contiguous with these cisternae. In addition, it is more common to find ALs in cells that have been exposed to cytotoxic compounds and viral infections.

This report addresses the unusual morphology of AL structure found in the hepatocytes of an electric ray while surveying the structure of its biliary tract (Gilloteaux et al., 1996). From our observations, we hypothesize that the presence of AL in hepatocytes could be an intracellular signal of sea pollutant cytotoxicity.

Materials and methods

Samples of liver tissues of 5 specimens of *Torpedo marmorata* Risso, 17-32 cm in length (3 males and 2 females) were captured by trawler fishing with the boat Nereis II, at 35-40 m depth from the sandy seabed (along a NNW-SSE line between Cape Béart to Cape de l'Abeille, 1 nautical mile before the edge of the National Marine Reserve) in the Bay of Banyuls (Gulf of the Lion, Mediterranean Sea, France). The fish were brought to the Arago Marine Biological Station of the University of Paris, and within 1/2 hour of capture they were kept in aerated sea water. The electric rays were decapitated to prevent any changes in the liver due to using dissolved anesthetic and, after removal of other organs of interest,

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liver specimens were cut in 1 mm- thick slices and fixed promptly at laboratory temperature (15°C) for 10 min in 3% glutaraldehyde diluted by sea water, and buffered by 0.1 M cacodylate buffer. Fixative was then changed and specimens were immersed for another 2 hours at 4°C before they were washed by the buffer alone containing 30 gm/L sodium chloride and 2 gm sucrose. Tissues were postfixed with a 2% aqueous osmium tetraoxide solution for 2h duration. Tissue samples were then processed and embedded in PolyBed 812 (Polysciences, Warrington, PA). Thin sections were observed in a Jeol 101 transmission electron microscope.

Results

Torpedo display elongated, prismatic and polyhedral shaped hepatocytes from 15 to 25 µm in length and 5 to 8 µm in width, and 6 to 7 µm in height; they are mostly uninucleate and contain very large amounts of storage glycogen as well as lipid droplets with dense bodies and yolk deposits. The hepatocytes reveal irregular and thick, microvilli extensions on their surfaces in the space of Disse (Fig. 1). A narrow 1.0 to 2.5 µm -thick bordering region of cytoplasm, rich in branching smooth endoplasmic reticulum, contains scattered mitochondrial profiles and bundles of actin-like filaments. These organelles appear to be confined to the encompassing cytoplasm among these filaments. Mitochondria, oblong to round shaped (1.5 µm long and 0.3-0.5 µm in diameter) display exaggerated electron densities, a poorly developed matrix, appears somewhat vacuolated and sometimes display minute whorls.

The unique observation made throughout the 5 liver specimens of this selachian is the presence of unusual circular ALs surrounded by glycogen and lipid deposits. In addition, pieces (?) or linear segments of AL can be detected near the circular AL and at the edge of these storage components. The large glycogen storages typically segregate other organelles as expected in the hepatocytes. However, the glycogen aggregates appear spread as a dense network instead of rosettes as viewed in a more classic macromolecular storage. In addition, within the glycogen storage component, lysosomal (?) whorls of membrane appear as onion bodies. This type of AL has unique, ultrastructural features, again because it is circular and it comprises two concentric rings of nuclear-like pore complexes each sandwiched between two narrow smooth endoplasmic reticulum (SER) cisternae. The innermost and outermost SER cisterns are contiguous and continuous with some SER tubular cross-sections outside the ALs; these are also adjacent to the main glycogen deposits of the central storage area of the hepatocyte. The inner ring contains a central area of cytoplasm filled with glycogen, lipid deposits, and smooth endoplasmic reticulum. A narrow cytoplasmic zone is sandwiched between the inner and the outer rings of AL-SER complexes and also contains sparse but delicate aggregates of glycogen-like storage.

Discussion

The intention of this report is not to describe the general architecture of the liver in this species that will be the object of another contribution but to describe a peculiar AL morphology present in this differentiated tissue. From these observations, the hepatocytes found in the Torpedo samples were of the same anatomical structure as that characterized by other works (Diaz and Connes, 1988; Rocha et al., 2001).

The name annulate lamellae (AL) varied early on, but the advances in electron microscopy allowed the study and description of AL occurrence, and their organization. However a full understanding of their tri-dimensional architecture as well as their function in cellular homeostasis needs further elucidation. In this report, we propose that the ALs observed originated from a spiral structure that could unwrap and dissect out small pieces, i.e. the segments found among the other organelles. The molecular organization of ALs has been related to the cell cycle and dissected by several groups (Dabauvalle and Scheer, 1991; Cordes et al., 1995, 1996; Meier et al., 1995; Dabauvalle et al., 1999; Beckheling et al., 2003). These recent publications help us to understand the early studies reporting that AL usually belong to cells that have active cell cycles or are prepared to undergo mitosis, such as animal and human cell lines (Wischnitzer, 1970; Kessel, 1983, 1992), including gametes or early division after fertilization (Billard 1984; Romeo et al., 1992), embryonic cells (Benzo, 1972, 1974), and pathologic ones (Kato et al., 2001), including injured liver cells (Hruban et al., 1965a,b; Kohda et al., 1989), hepatomas and other tumors (Hoshino, 1963; Svoboda, 1964; Ma and Webber, 1966; Locker et al., 1969; Kim et al., 1990; Eyden, 2000; Biernat et al., 2001) and in virus-infected cells (Marshall et al., 1996). Additionally, recent data confirmed that ALs are associated with the complex control and regulation of the initial steps leading to the pre-mitotic phase of the cell cycle (Cordes et al., 1996; Beckheling et al., 2003), including the disassembly and reassembly of the nuclear envelope (Erlandson and De Harven, 1971; Meier et al., 1995; Cordes et al., 1996).

Furthermore, the presence of glycogen and lipids in these hepatocytes is not a surprise as this organ is the main reserve storage of glycogen and this storage favors segregation of organelles (Bruslé and González I Anadon (1996). Even though we managed to preserve the tissue as soon as the fish was captured, and according to previous literature (Spielberg et al., 1993), one observed the glycogen clumped into loose aggregates, not forming the rosettes morphology as classically described and detected many droplets of fatty deposits found throughout hepatocytes were well preserved. As in other selachians, the lipid reserve is rich in squalene and dense bodies (Peyronel et al., 1984; Rocha et al., 2001; Sarasquete et al., 2002) that could make the liver cells more susceptible to lipophilic toxic

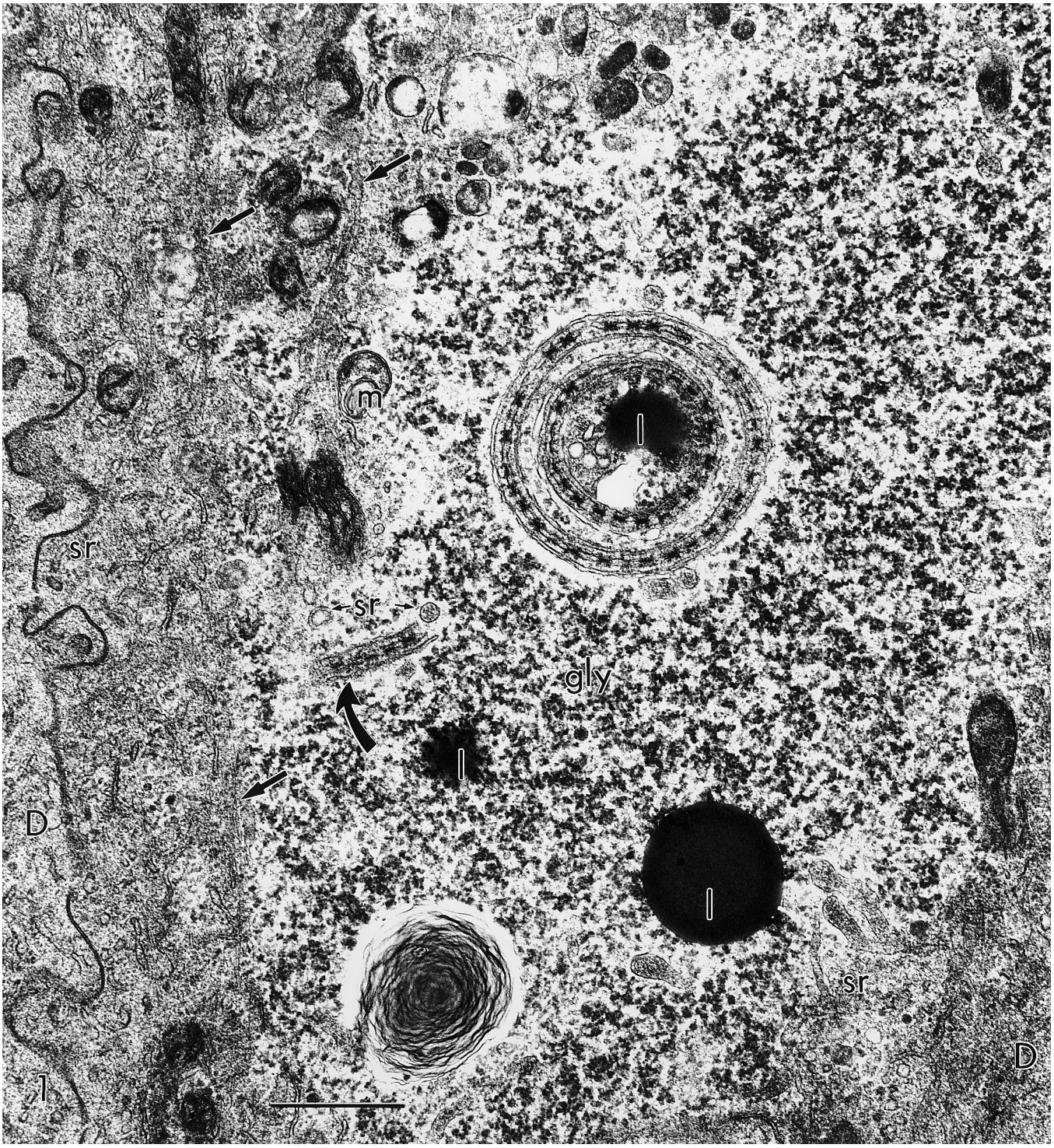

Fig. 1. Example of an hepatocyte content from *Torpedo marmorata* showing one annulate lamellae (AL) complex in a glycogen- and lipid-rich cytoplasmic area. A fragment of AL (curved arrow) is also shown. Bundles of actin-like filaments are indicated (straight arrows) among the smooth endoplasmic reticulum (sr). D: space of Disse; gly: glycogen deposits; l: lipid droplet; m: mitochondria. Bar scale: 1 µm.

compounds that would induce membranous whorls or onion bodies. Five specimens captured were within the size expected (Filiz and Mater, 2002) and showed nor gross neighter histopathologic anomalies in their livers. It was only because the biliary tract was studied with the electron microscope that circular ALs were detected in the hepatocytes.

The mere presence of peculiar ALs in differentiated hepatocytes co-localized with cytoplasmic whorls or onion bodies and the simultaneous finding of adjacent damaged mitochondria and dilated smooth endoplasmic reticulum with an altered pattern of glycogen aggregates led us to suspect that this could signal intrahepatic cytotoxic subcellular damage or sensitization to local shoreline or sea effluent effect reaching the sea floor and affecting *Torpedo*, a bottom feeder and predator.

These changes are not different to those observations made in the hepatocytes of several freshwater and sea water fish by various research groups showing lesions in ER, lysosomes, ALs and other organelles (Klaunig et al., 1979; Van der Heijden and Dormans, 1981; Hinton et al., 1984; Braunbeck and Völkl, 1991; Arnold et al., 1996; Zahn et al., 1996; Braunbeck and Applebaum, 1999). These changes were confirmed by the works of Biagianti-Risbourg (1990), Klaunig et al. (1979), Hinton et al. (1984) and reviewed by Bruslé and González-Anadon (1996) refer to the same cytological lesions detected in hepatocytes of other fish species. These changes were also observed as this was found in other fish and those found in a mammalian model treated by a toxic compound (Kohda et al., 1989).

Even though these ALs were observed in a small number of specimens, as these fish were collected along with another series of bottom feeder species for a first description of the hepato-biliary system (Gilloteaux et al., 1996), we believe that the finding of this observation of ALs of peculiar shape in this selachian liver cells was of interest to be reported as an initial cytological marker for cytotoxicity and to maybe grant further investigations related to biomarine welfare.

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