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患鰓腎炎養殖鰻 (*Anguilla japonica*) 之腎小管上皮細胞內玻璃球體之超微結構

Ultrastructure of the Hyaline Granules in the Renal Epithelial Cell of Cultured Eel (*Anguilla japonica*) Associated with Branchionephritis

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Abstract

The renal epithelial cells of cultured eels which showed symptoms of branchionephritis were studied with electron microscopy. The general structure of the epithelial cells was described with special emphasis on the single membrane bound electron dense granules which were corresponded to the hyaline granules observed under light microscopy. Two types of electron dense granules were revealed according to their internal structure. Type I granule had amorphous electron dense matrix within. Type II granule had a few to over a dozen of membraneous whorls. These granules were comparable to derobodies and residual bodies morphologically. The releasing of those electron dense granules into the tubules was associated with cellular degeneration and necrosis under severe pathological development.

Introduction

Branchionephritis in cultured eels were reported on several occassions[1,2,9]. This disease also had been observed in Taiwan in recent years[1,9]. The causal agent of this disease is not known. It occurred during the winter months when the water temperature was low. The histopathology of the internal organs of the diseased fish was first reported in 1970[1]. In general, the pathological symptoms can be readily recognized both in its gill and kidney. In the diseased fish, its abdomen appeared depressed and the gill laminae became club-like. Necrosis of the renal glomerulus and the appearance of the hyaline granules of various sizes in the renal epithelial cells and later in the urine were reported[1,9,10]. Neither the ultrastructure nor the origin of these granules were examined. It seems that ultrastructural studies of the epithelial cells of the renal tubules and of the hyaline granules would be helpful to our understanding of this disease.

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This report presents some of the observations on the ultrastructure of the epithelial cells of the renal tubules. Emphasis will be made on the morphology of the hyaline granules.

Materials and Methods

Renal tissues from eels with branchionephritis were cut into small pieces and fixed with 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.6 for one and one half hours. After being washed for a few times with the same buffer the tissue blocks were post-fixed with 1% osmic acid in the 0.1 M phosphate buffer for one and one half hours. After rinsed with the same buffer they were dehydrated with ethanol, treated with propylene oxide and embedded in Epon and Araldate mixture.

The samples were sectioned with a diamond knife on a LKB ultramicrotome. Thin sections of less than 100 nM were picked up on 300 mesh cupper grids. After being stained with uranyl acetate and lead citrate the sections were observed with a JOEL 100 CX electron microscope at 60 KV.

Results and Discussions

The renal tubules of the teleosts vary in length and appearance. Generally it has a ciliated neck segment, a bisegmental proximal segment, a ciliated intermediate, and a distal segment which open into the collecting duct[3,5,6,10,11]. In this study, judged by the shape of the epithelial cells, the surface specialization and the cellular organelles several segments were studied.

The neck segment is characterized by cuboidal epithelium[3,5,11], with numerous long cilia (Fig. 1). The function of this segment is uncertain. It is thought that the ciliary action has some importance in promoting the flowing rate of the fluid along the tubules[11].

The lining cells of the first proximal segment are cuboidal or low columnar with welldeveloped brush border (Fig. 3), lysosome system and numerous mitochondria. This segment probably functions in glucose, sodium and chloride reabsorption and is capable of active secretion of organic anion[3,5,11]. The second proximal segment is the predominant segment in teleost fish. In this region the brush border of the epithelial cells is not as well developed as in the first segment. The cells are tall columnar, with numerous mitochondria (Figs. 2 and 3). The main function of this segment is organic anion and cation secretion and in divalent cation transport[11]. The nuclei of the epithelial cells were prominant, and were round or irregular in shape. Most of the nuclear area were occupied by euchromatin and very little heterochromatin was observed (Fig. 3). This suggested that these cells were actively engaged in metabolic activities. Some of the mitochondria were swollen and globular in shape, and matrix and cristae were partially dissolved (Fig. 3).

The intermediate segment follows the second proximal segment. It is known only in freshwater fish. The structure of the cell is similar to the neck segment by having ciliated cuboidal or low columnar epithelial cells[5,11]. The function of this segment is thought in propelling the fluid along the tubule[11].

The distal section of the renal tubule resembles that of higher vertebrates such as am-

phibians, birds and mammals, being composed of columnar cells with numerous mitochondria arranged perpendicular to the basement membrane[11].

In the proximal tubules the deep inveginations of the cytoplasmic membrane was evident with broken end, and the mitochondria were round or irregular in shape (Fig. 2), which was different from the longitudinal, orderly arrangement under normal condition. The moderate alternations of the membrane integrity and the shape of the mitochondria may represent the on setting stage of the pathological effects of branchionephritis. Electron dense granules were observed intracellularlly and extracellularlly in the lumen of the tubules (Figs. 1, 3, 5 and 6). The size of these granules vary greatly, they were ranged from 0.2 to 2 μ in diameter. These electron granules were identical to the hyaline granules observed with light microscopy[1,8,9].

These granules were enclosed in an unit membrane. According to their morphology and content two types of electron dense granules were recognized. Type I granule had amorphous electron dense matrix within and were usually smaller in size (Figs. 1 and 4). Type II granule contain several membraneous whorls. The number of the whorls varied from a few to more than a dozen in the section of a single granule (Figs. 4 and 6). These were morphologically similar to the residual bodies of cytolysosome in the cytoplasm and found in tumor cell[4,7].

Some of the tyrpe I granules were morphologically similar to the mitochondria in the renal epithelial cell of flounder after exposed to 10^{-4} M mercuric chloride for 4 hours[11]. Similar membrane bound electron dense granules were observed in Fleischmann baker's yeast cells after thallium oxide, thallium sulfate or cadmium nitrate were added in the culture medium[8]. Those electron dense granules were morphologically similar to type I electron dense granules described in this study. The similarity between these granules observed in renal tubular cells and yeast cells suggested that the contamination of heavy metal ions of the culture ponds should be suspected. Oka *et al.*[9] also had suspected that the inbalance on accummulation of metal ions in the epithelial cells coupled with the decrease in temperature may be responsible for the formation of hyaline bodies in those cells.

中 文 摘 要

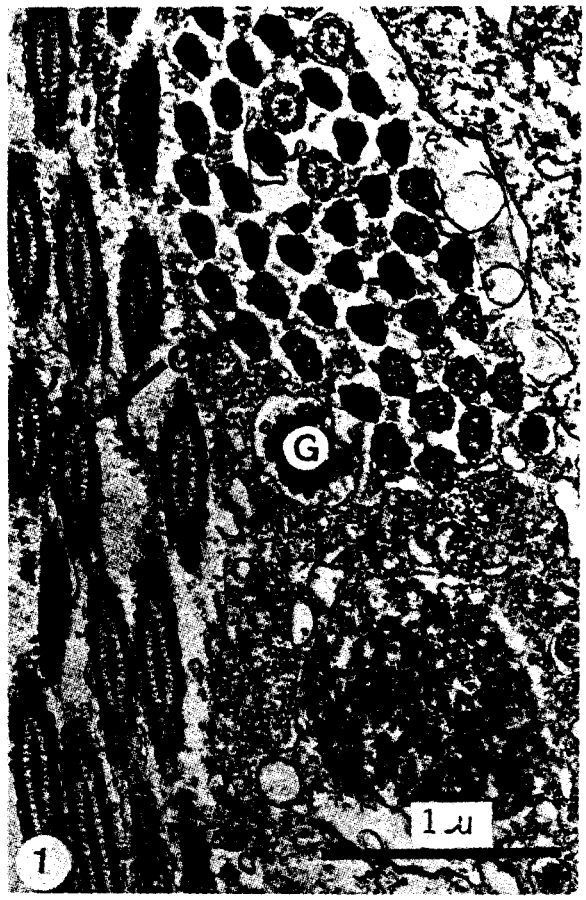
本文描述患鯉腎炎病鰻的腎小管上皮細胞內玻璃球體之超微結構。玻璃狀小球外覆被膜，內含不明之物質，以內部物質的形態可分爲兩類小球，第一類具顆粒狀內含物，第二類具多重膜狀內含物。前者類似生長在含重金屬培養基中酵母菌細胞內的後生體，及暴露於含有氯化汞水中比目魚腎小管內上皮細

Legend

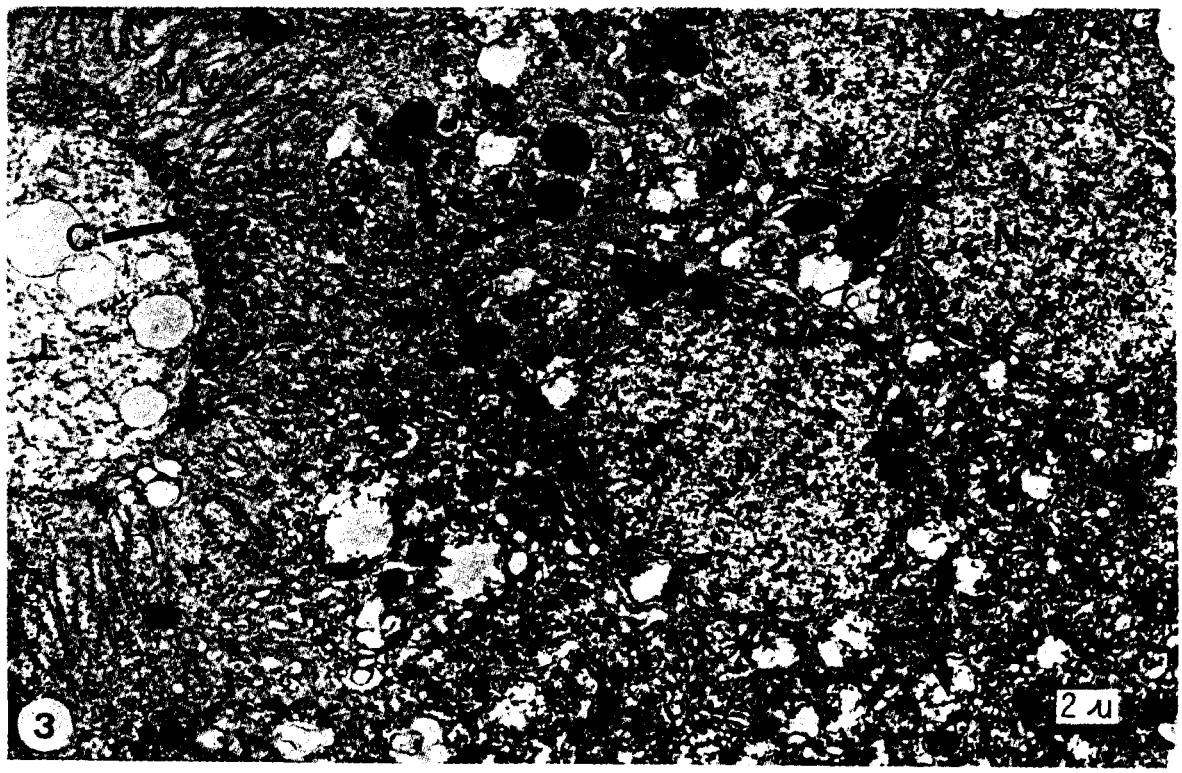
Fig. 1. Higher magnification of cilia (C) in cross and oblique section, and electron dense granules (G) with single bounding membrane (arrow head). ×28,000

Fig. 2. Electron micrograph of the proximal tubule showed structural altered mitochondria (M), and broken ends of the cytoplasmic membrane inveginations (arrow head). ×16,000

Fig. 3. Electron micrograph of the renal tubule of cultured eels associated with branchionephritis, tubular lumen (L), cilia (C), microvilli (MV), nucleus (N), structural altered mitochondria (M) and electron dense granules (G) were identified. ×5,500


G
1μ
1


1μ
2


2μ
3


G1
M
G2
1 μ
4


2 μ
5


1 μ
6

胞中之粒線體；而後者則酷似溶素體之殘餘體。腎小管上皮細胞壞死後玻璃狀體小球及部分細胞質脫落於管腔中。

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Legend

Fig. 4. Higher magnification of intracellular electron dense granule Type I (G1), Type II (G2) and structural damaged mitochondrion (M) still having intact double (arrow). ×25,000

Fig. 5. Higher magnification of extracellular electron dense granules Type II (G2) which corresponded to the hyaline granules under light microscope. ×35,000

Fig. 6. Shadding epithelial cytoplasm from the renal tubule at the later stage of branchionephritis, nucleus (N), intra and extracellular mitochondria (M) and granules (G) were identified. ×18,000