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LIGHT AND ELECTRON MICROSCOPIC OBSERVATION OF THE GRANULOMATOUS TISSUE OF REARED CATFISH, PARASILUROUS ASOTUS

光學與電子顯微鏡對於養殖鯰魚肉芽腫瘤組織的觀察

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Abstract

Granulomatous outgrowth on the body surface and the internal organs were observed of reared catfish. Histologically, the granulomatous tissue consists of multicellular giant cell. Among the granulomatous tissue investigated, two categories of giant cells were observed underlight microscope. One kind of the giant cells showed structural zonation: with a central amorphous central core, a transitional zone and a peripheral cellular zone. The other kind of giant cell was a multicellular symplasm without structural zonation. The structral characteristics of each zone of the first kind of giant cell and the difference between the two kinds of giant cells were studied both on light and electron microscopic level. The possible causal factors of the granulomatous development were also discussed.

Introduction

Histologically, granuloma is resulted by giant cell formation, which is a body defense reaction towards the invasin of materials which may either biological or nonbiological; either endogenous or exogenous origin. (Dumont & Sheldon, 1965; Frankal *et al.*, 1962; Papadimitriou & Archer, 1974; Smith & Davis, 1971; Spector et al., 1970; Wolf & Jackson 1963). In the literature, reports on granulomatous studies can be traced back to the late period of 19 centry (Patek & Bernick. 1960). In higher vertebrates, both in vitro and in vivo studies demonstrated that the formation of giant cell is due to the fusion of phagocytic cells (Carter & Rebert, 1931; Sulton & Weiss 1966; Weiss & Fawcett, 1953; Weiss, 1974). Morphological and structural studies of phagocytic cells and giant cells were also reported (Dumont & Sheldon, 1965; Papadimitriou & Archer, 1974; Sulton & Weiss, 1966; Weiss, 1974).

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In fish granulomatous outgrowth were reported in several species of trout (Dunber & Herman, 1971; Wolf & Jackson, 1963). In 1980, a systemic granuloma in *Sparus surata* cultures in the Gulf of Aqaba was reported (Papaerna *et al.*, 1980). In the summer of 1982 and 1983, at the harvesting time of cultured catfish, *Parasilurus asotus* in Lukang Branch, Taiwan Research Institute, granulomatous protrusions were observed among some of the fish, both on the external part of the body and among the internal organs (Chien & Yu, 1983). This report is mainly an electron microscopic study of the giant cells of the granulomatous tissue of the reared catfish.

Materials and Methods

Tissue pieces were obtained from the trunk kidney and other organs. The sample pieces were cut into small blocks and were processed for electron and light microscopic study. They were fixed in 3% gluteraldehyde in phosphate buffer at pH 7.3 for two hours and post-fixed in 1% osmic acid in the same buffer for two hours. After dehydration in increasing concentration of ethonal and treated in propylene oxide, the sample blocks were embedded in Epon and Araldite mixtnre. After polymerization, thin sections for electron microscopic study were cut with glass knives on LKB ultramicrotome and double stained with uranyl acetate and lead citrate. Observations and photoghaphs were made on a JEOL 100CX electron microscope at 60 to 80 KV.

Thick sections were cut from the same blocks as for thin sections for light microscopic observation.

Results

Granulomatous tissues observed both on the external surface and in the internal organs such as the heart, liver, muscle and kidney. They were widely distributed and different in size ranged from sized protrutions to sandy grain sized white spots. Among the internal organs, kidney was ranged the most severe organ with granulomatous tissue formation. A general survey of the distribution and apperance of the granulomatous tissue formation is presented in table 1.

Table 1. Apperance and locations of the granulomatous tissues on the body surface and internal organs of the catfish Name of organs

	General apperance of the granulomatous tissue
Barbel base	Nodule to protrusion
Maxilla	Nodule to protrusion
Base of annal fin	Nodule to protrusion
Heart	Whitish, sandy grain
Liver	Whitish, sandy grain
Kidney	Whitish, sandy grain
Skeletal muscle	Small nodule to sandy grain
Gill racker	Small nodule

Under light microscope, the granulomatous tissue was a accumulation of many multicleated cells. Most of the multinucleated giant cells were spherical in general, some of them were irregnlar in shape. Most of them were having an amorphous core of granular in textnre, and a pheripheral zone where showed different in density, from area to area and nucleus and cytoplasm were distinguishable. The size of the central core relative to the size of the giant is different from giant cell to giant cell. Another kind of giant cell is just a symplasm without central amorphous core (Fig. 1, 2, 3).

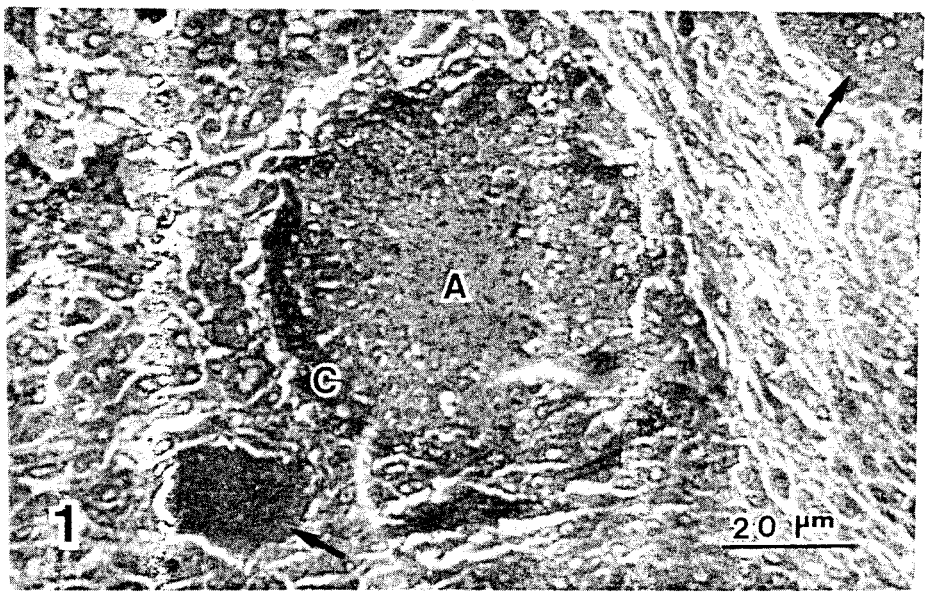

Figure 1. Giant cell from the granulomatous tissue of reared catfish. The giant cell showed a central amorphous core (A), peripheral cellular zone (C), and the spindle shaped phagocytic cells around the giant cell will become part of the giant cell eventually. Some other giant cells are also show (Arrows).

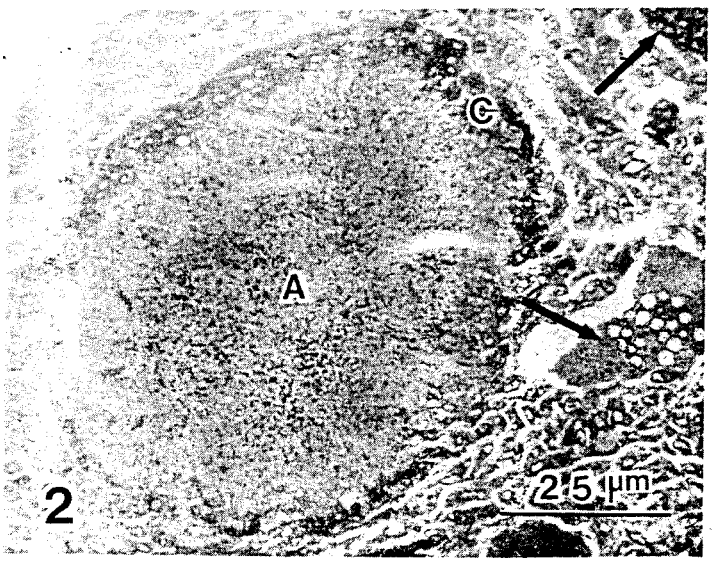

Figure 2. A large granulomatous giant cell with a large amorphous central core (A), and a peripheral cellular zone (C), and parts of other giant cells (Arrows) are shown around the large giant cell. The phagocytic cells around the large giant cell will eventually fuse with the large giant cell as the giant cell developes.

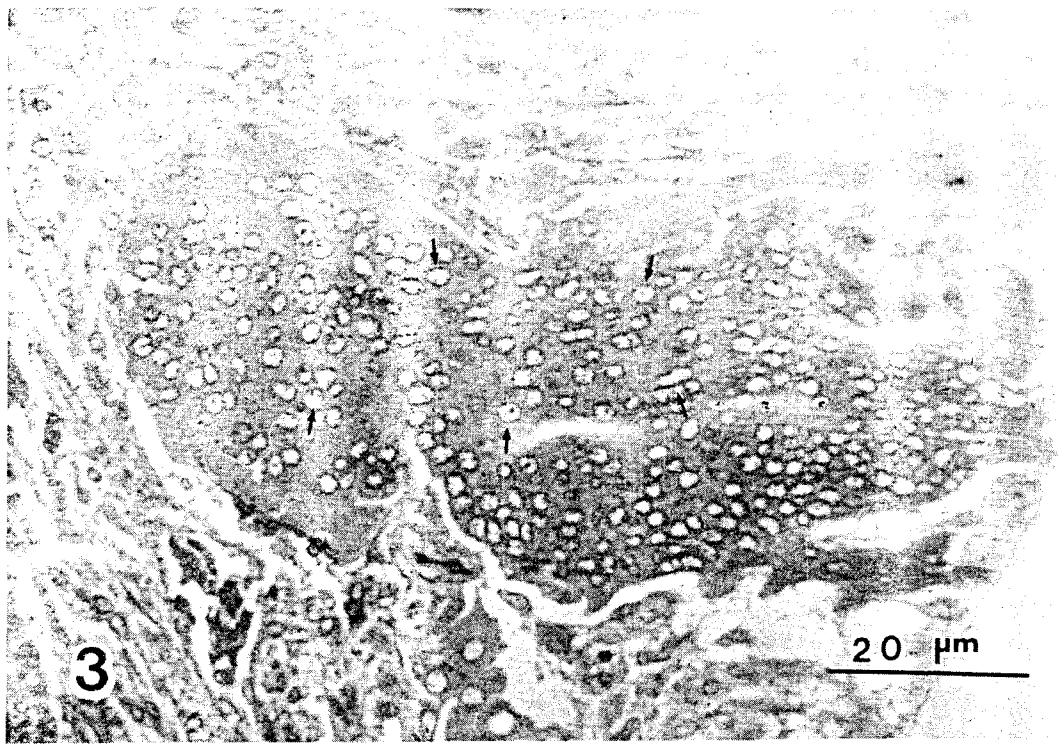

Figure 3. Giant cell from the granulomatous tissue of reared catfish kidney. Figure shows a symplasmic giant cell without central amorphous core, having numerous nuclei (Arrows).

Electron microscopic observation of the giant cells

1. Giant cell with amorphous central core

Under electon microscope the giant cell with amorphous central amorphous core, according to the composition differences three zones can be localized: central amorphous core, transitional zone and peripheral zone. For the convenience of presentation, each zone is discussed separately.

A. Amorphous central core

The central core of a giant cell occupied relatively the central part of the giant cell. Under electron microscope this region consisted of condensation of materials of cellular origin but no identifiable structure known of a common cell. The central area were deposit of vesicles, electron dense material and structures similar to myelin figure (Fig. 4, 5).

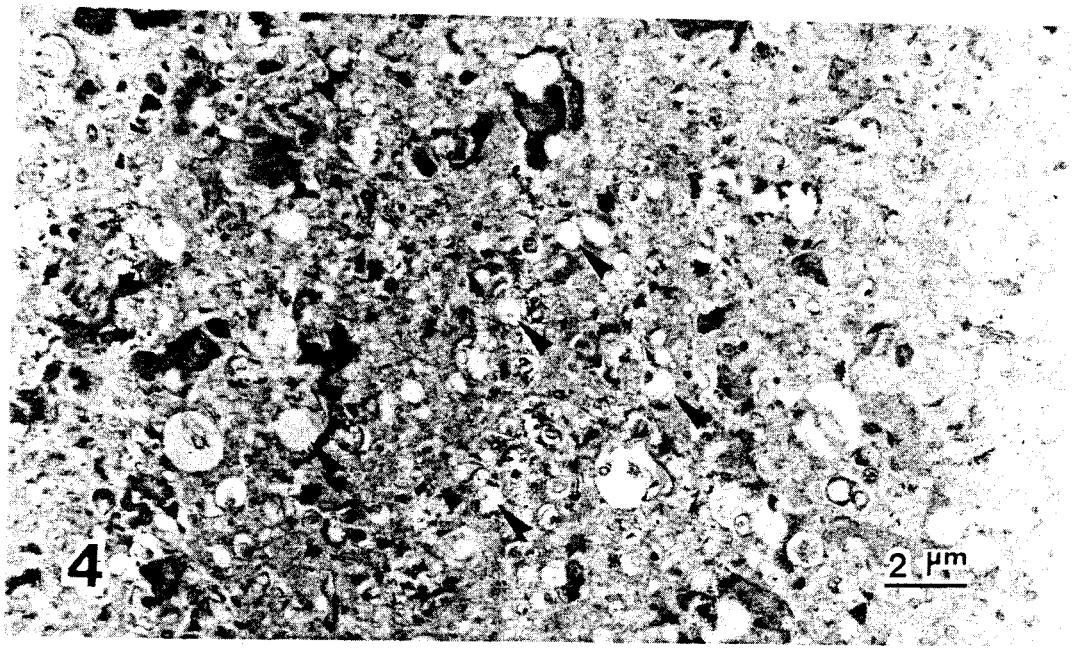

Figure 4. Lower magnification of the central amorphous core actually consists of materials of different density and vesicles (Arrows).

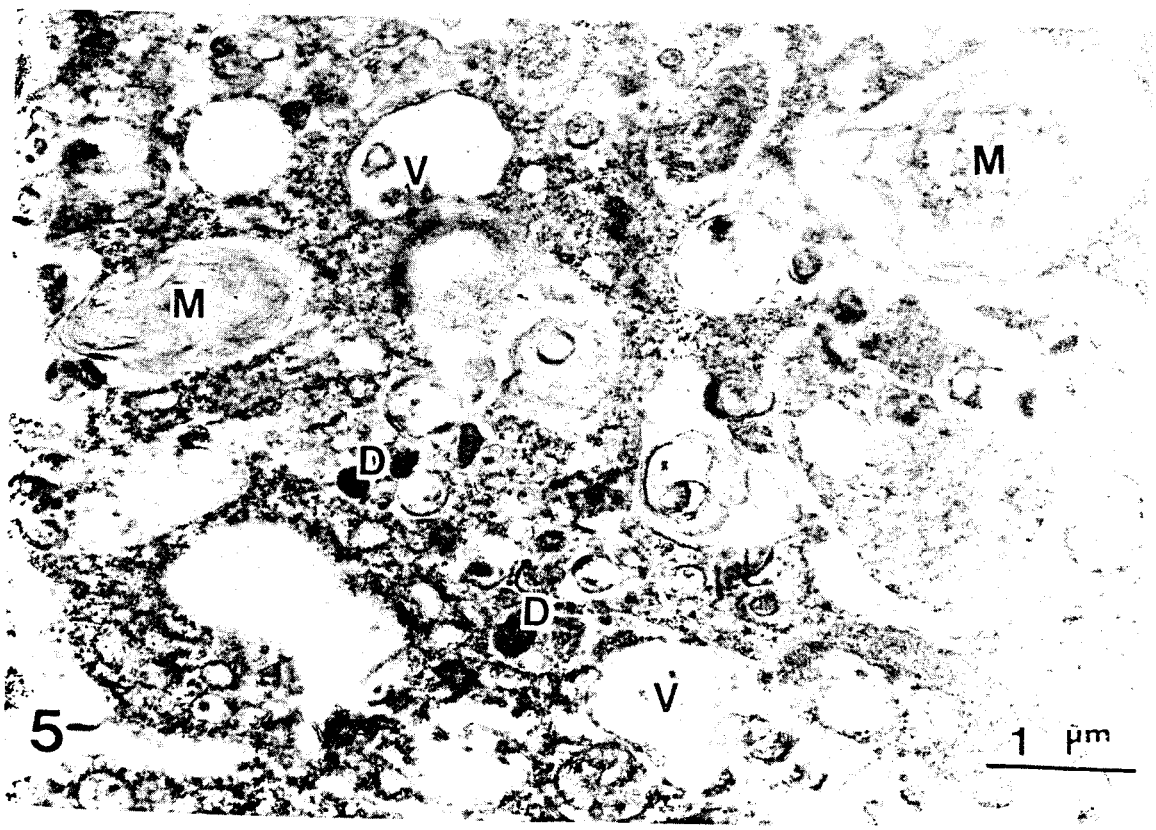

Figure 5. Higher magnification of the amorphous central core observed under light microscope. The central area is actually accumulation of cellular material. There are vesicles (V), myelin figure (M), dense bodies (D) and many granular structures.

B. Transitional zone

As the field of observation moved from the central amorphous core towards the peripheral region, the amorphous material became less densely packed and distingishable cellular organelles such as ribsomes and nuclei were observed. Also there were many inclusion bodies with contents similar to those of the central amorphous core in apperance (Fig. 6). The nuclei were in different functional conditions. Some of the cells were still normal in apperence with intact membrane, nuclear pores and nucleolus. Some of them were in advance degenerational stage. Mitochondrion was not as frequently observed as nucleus.

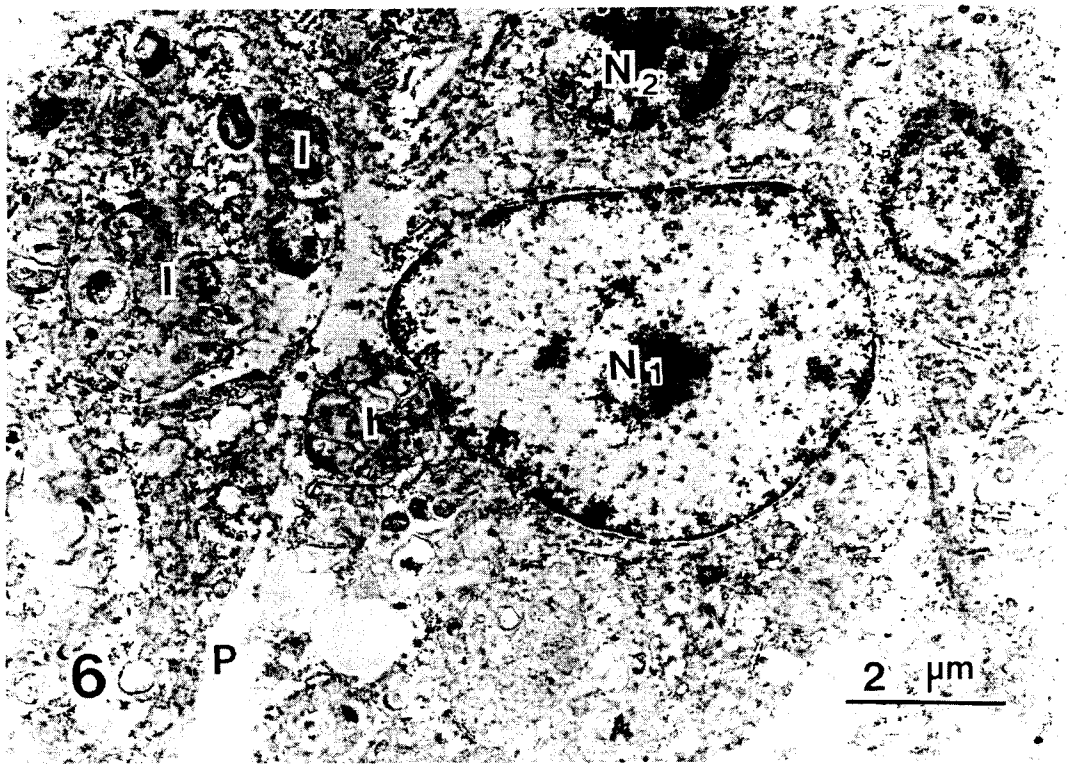

Figure 6. Electronmicrograph from the transitional zone of a giant cell of the granulomatous tissue. Nucleus 1 (N1) is still at normal condition but nucleus (N2) was degenerated in apperance. Inclusion bodies (I) with content similar to these of the central core. The whole area was loosely packed with large intragiant cell spaces (P).

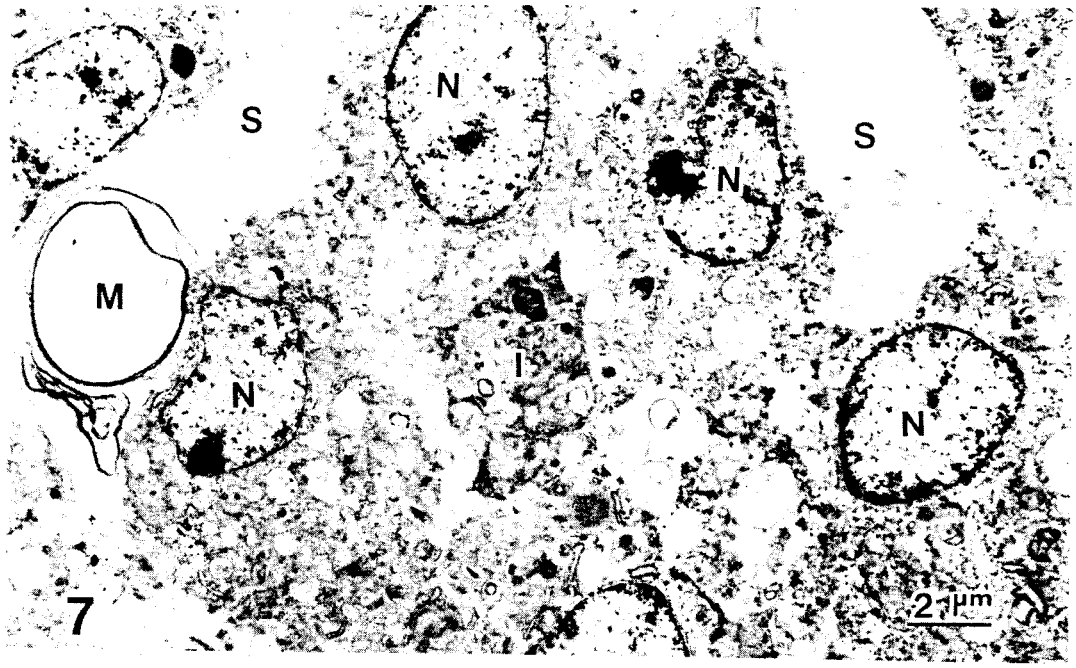

Figure 7. Electronmicrograph from the transitional zone to show the combined nature of this zone which contained many nuclei (N) indicated the fusion of many cells and inclusion bodies similar to the apperance of the central core (I), formation of myelin figure (M) and large intracellular space (S).

C. Peripheral cellular zone

As the field of observation moved from the transitional zone towards the outskirts, there were cells partially jointed to the giant cell partially kept free and separated from the giant cell (Fig. 7, 8). There were large intercellular spaces between each separated portion, and the cell membrane was not clearly shown. The cytoplasm contained many inclusion bodies (Fig. 8).

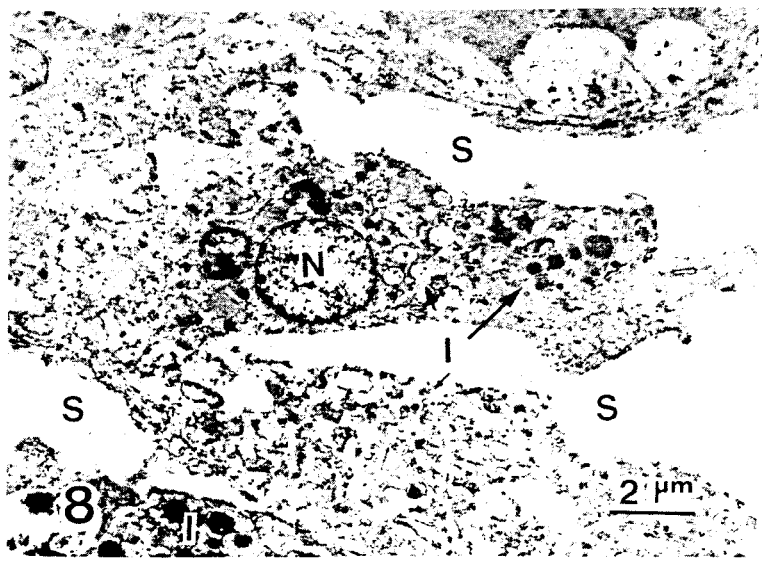

Figure 8. Electronmicrograph of the cellular zone of the giant cell. It shows three partially connected cells with larrge intercellular spaces (S), one of the nucleus is shown (N), many inclusion bodies (I) indicats these cells are in the processes of intracellular digestion and degeneration.

2. Samplasmic giant cell without central core

Under light microscope this type of giant cell were vary in size. Some of them contained a few nuclei and some of them contained hundreds of nuclei in section profile (Fig. 3). The nuclei were evenly distributed, there was no distingishable regional difference. Under electron microscope, the cytoplasm was more or less homogenous in nature. The predominant cellular organelle was rough endoplasmic reticulum. The nuclei were irregular in shape and varied in size (Fig. 9). Lysosomes and inclusion bodies were also noticed but were relatively fewer in number and smaller in size as compared with that of the giant cell with central core.

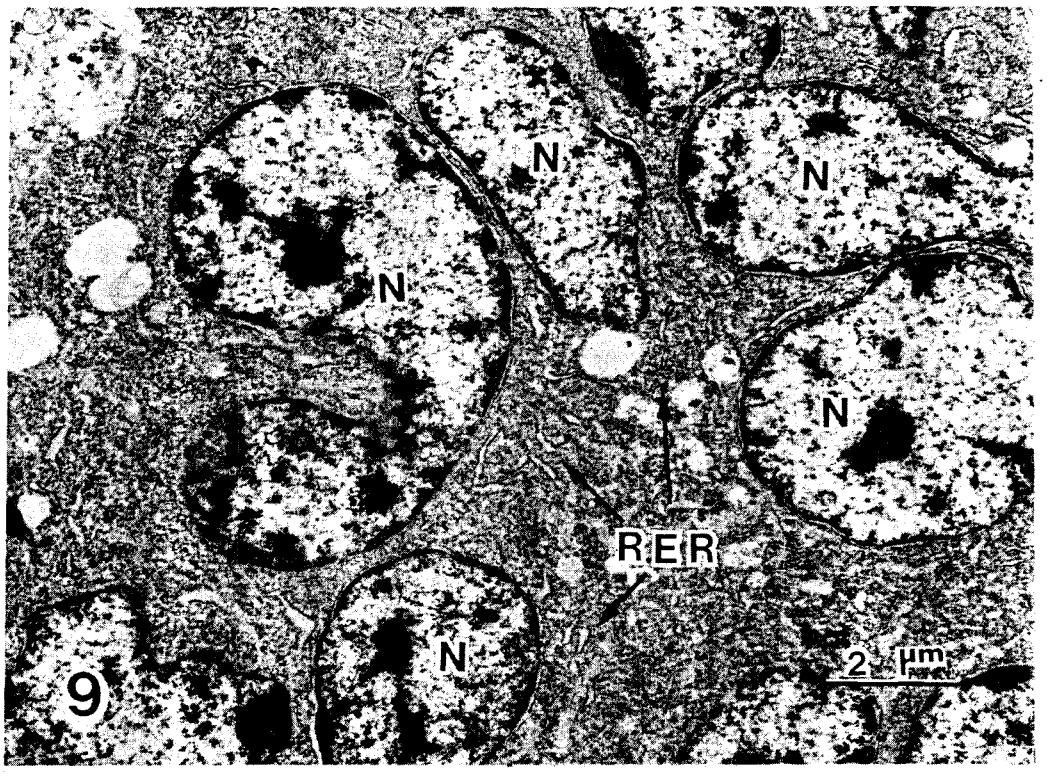

Figure 9. Electronmicrograph of part of the giant cell without central core. Nuclei (N) of different size and shape and rough endoplasmic reticulum (RER) were the predominant organelles.

Discussion

The function of the phagocytic cell which includes polymorphonuclear leukocytes, kupffer cells of the liver, glial cells of the central nervous system, dust cells of the lung, as well as the histocyte of the connective tissue, is body defense against the presence of particles of biological or non-biological either exogenous or endogenous origin (Cohn & Wiener, 1963; Dumont & Sheldon, 1965; Frankal *et al.*, 1962; Papadimitriou & Archer, 1974; Patek & Bernick, 1960; Smith & Davis, 1971; Spector *et al.*, 1970; Wolf & Jackson, 1963). Phagocytosis and intracellular digestion by lysosomal enzymes are the fundimental mechsisms in accomplishing their defensive reponsibility (Cohn & Benson, 1965; Cohn & Wiener, 1963; Spector *et al.*, 1970; Weiss &

Fawcett, 1953). At times if the particles are not eliminated effectively and more phagocytic cells are attracted to the site. A chain rection of fusion and accumulation of the cellular debris is induced and it results giant cell granulomatous tissue formation (Cohn & Benson, 1965; Cohn & Wiener, 1963; Spector *et al.*, 1970; Weiss & Fawcett, 1953), The systemic granulomatous tissue formation both in the surface and the internal organs observed in catfish should be results of the same reaction yet the causal material if systemic presence over the entire body system even the nature of this material is not known at this time.

The processes of giant cell formation have been interested by many researchers, and many cell types and methods to induce giant cell formation have be tried (Carter & Robert, 1931; Papadimitriou & Archer, 1974; Papadimitriou& Wyche, 1974; Patek & Bernick, 1960; Smith & Davis, 1971). The early literature of giant cell formation by tissue culture can be traced back to 1912 (Papadimitriou & Wyche, 1974). and many different sources of materials: asbestos (Smith & Davis, 1971). india ink (Patek & Bernick, 1960). nitrosoquinoline (Carter & Robert, 1931). zymesan and throtrast (Weiss, 1974) injection; implantation of large particles (Papadimitriou & Archer, 1974; Papadimitriou & Wyche, 1974). These efforts reflected the importance of the fundimental mechanism of self defense. Nevertheless most of the studies were conducted with high vertebrates. Using carrageenin inducing granuloma was studied with plaice (Papadimitriou & Archer, 1974; Papadimitriou & Wyche 1974). Granuloma observed in fish were associated with trout under cultural condition (Dunber & Herman, 1971; Wolf & Jackson, 1963). The causal agent was finalized to the cotton seed meal in feed. Metabolic inbalance induced the action of phagocytic cell and resulted granuloma formation. Also high water temperature was found related to the development of the epizootic.

In brook trout not only the feed containing cotton seed meal but also was found that the high water temperature was related with visceral granuloma formation (Dunber & Herman, 1971). The catfish reported in this report were on eel feed. The particular ingradient which was responsible for the systemic granuloma formation is not specified yet, but the high subtropic temperature may also related to their development. Besides no bacterial or viral was observed in the granulomatous tissue. No mortality was observed.

It was reported that a systemic granuloma in Sparus aurata caused losses of fish in the period between March and October indicated the development of the disease associated with higher temperature, also diet containing fish meal was found associated with epizootic of this disease. It seems that cultural condition, imbalance of diet and higher water temperature are related to the development of fish disease among fish. In this study, we present a ultrastructural picture of the sequential change from phagocytic cells to the giant cell and eventually degradation. to unidentifiable cellular debris.

The giant cell without central core may be one of the two cases. First, it may represent a just form young giant cell in the early developmented stage, or a section only through the peripherial cellular zone. The age of a giant cell may be sought from the size or proportion of the central core to the peripheral cellular zone.

中 文 摘 要

養殖鯰魚在收穫時發現在體表及解剖後的內部器管有肉芽腫瘤的發生。此種肉芽腫瘤爲由若干性細胞結合成的肉芽性巨形細胞所構成。觀察中發現肉芽性巨形細胞有兩類。一類爲圓形，中心部分爲酶解後細胞殘留物，由中心向外觀察則見尚未完全酶解後的細胞殘留物如細胞核及內質網等胞器。外層則可見到初步與巨細胞結合的吞噬性細胞。第二類爲不定形之多核原生質形的巨細胞，中心與周邊在結構上，無分化之現象。在本報告中兩類巨細胞的形態結構，在光學與電子顯微鏡的層次做了比較的觀察，也對關於可能引起肉芽腫瘤的原因做了討論。

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