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*Full Length Research Paper*

# Ultrastuctural study of the phagocytic activities of splenic macrophages in tilapia (*Oreochromis niloticus*)

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**The main stages of the phagocytic process after the intraperitoneal injection of colloidal carbon to tilapia (*Oreochromis niloticus*), was ultra-structurally documented. Carbon particles were initially in diffuse form in the cytoplasm but they gradually formed discrete electron dense bodies that later fused with cytoplasmic granules to form phagolysosomes. Ultra structural results demonstrate that colloidal carbon is actively phagocytized by cytoplasmic granules (lysosomes) in the spleen of *O. niloticus*. Direct phagocytic reaction of cells in the presence of an antigen is therefore very important in immunity.**

**Key words:** Tilapia, *Oreochromis niloticus*, ultrastructure, colloidal carbon, phagocytic activities.

## INTRODUCTION

Fish are one of the most primitive vertebrates and are an important link between invertebrates and higher vertebrates. They possess the non-specific defence mechanisms of the invertebrates such as the phagocytic mechanisms developed by macrophages and granular leucocytes, and were also the first animals to develop both cellular and humoral immune responses mediated by lymphocytes. The main lymphoid organs of fish are the thymus, the anterior kidney and the spleen. In fish, non-specific immunity is considered as the first line of defence and represents a considerable part of the immune response (Dalmo et al., 1997).

The innate (non-specific) immunity is thought to have a major role in disease resistance of fish (Roed et al., 1993; Balfry et al., 1997a,b). The most important cells involved in this defense are the phagocytes (Esteban and Meseguer, 1997; Esteban et al., 1998). Phagocytosis is a defense mechanism against infection common to all metazoans, including invertebrates. The phagocytic system in fish consists of resident macrophages present in all organs and tissues of the body. They are the first phagocytes to encounter the invading microorganisms and as such, play a very important role in the initial stages of infections.

Phagocytic activity is an important immunological parameter in the study of bacterial infections in fish, since it is related to the nutritional status, age and race of the animals and to preventive or therapeutic treatment. Several parameters of the innate immune response, such as respiratory burst activity, spontaneous haemolytic activity, lysozyme activity, complement concentration, and total IgM, are found to be associated with disease resistance in fish (Wiegertjes et al., 1996).

The aim of the present work was to study the phagocytic response of splenic macrophages to intra-peritoneal injection of colloidal carbon in tilapia (*Oreochromis niloticus*).

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## MATERIALS AND METHODS

### Fish

Twenty (20) juvenile tilapia (*0. niloticus*) weighing between 45.5

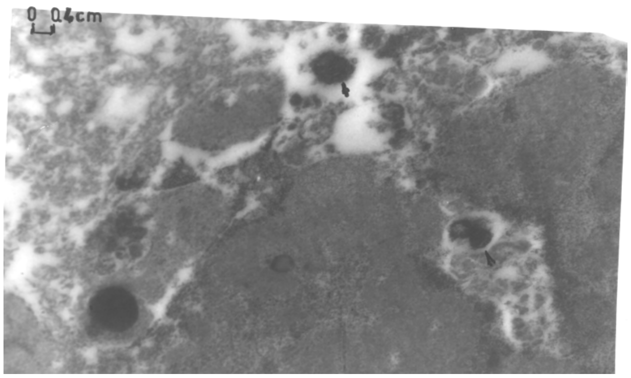


**Figure 1.** Electron micrograph of an ellipsoid wall (EW) with a carbon laden macrophage closely applied. Note the carbon particles within the cytoplasm. X 8000 (Let X 2000= 0.1cm, Scale bar = 0.4cm)

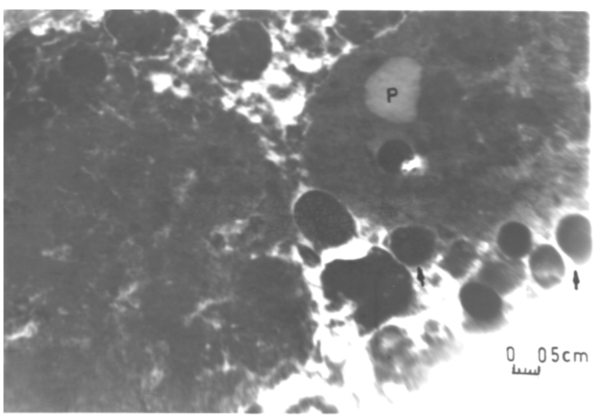


**Figure 2.** Electron micrograph of a macrophage with carbon particles diffusely scattered within the cytoplasm. X 10000 (Let X 2000= 0.1cm, Scale bar = 0.5cm)

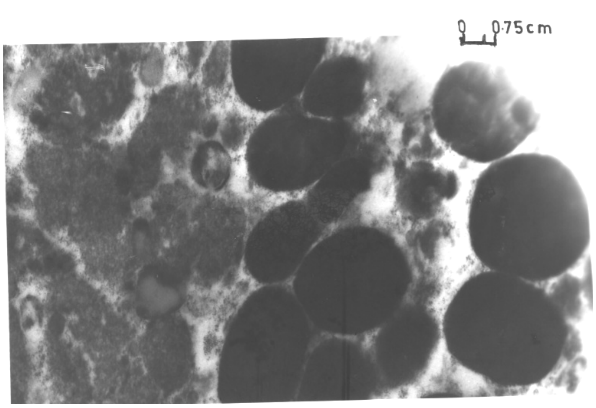


**Figure 3.** Aggregation of phagocytosed free carbon particles formed discrete electron dense bodies (arrow) X 15000. (Let X 2000= 0.1cm, Scale bar = 0.75cm).

and 60.2 g were purchased from a fish farm (Zartech farms, Ibadan). Clinical, parasitological and bacteriological examinations showed the fish to be free of infectious diseases. The fish were acclimatized at 24.5-25.5°C for fourteen days and fed with commercial fish pellet at 4% body weight. The natural photoperiod was maintained during the acclimation and experimental periods.

**Preparation and injection of carbon**

A 50% suspension of Rowney Kandahar 28 black Indian ink was prepared in distilled water. Fish, were lightly anaesthesized with 0.2 mg benzocaine dissolved in 5 ml of acetone and added to 8 litres of water (Roberts, 1978). In the test group, each fish was injected intraperitoneally with 0.2 ml of the carbon suspension. Control fish were injected with 0.2 ml of distilled water. Two (2) test fish and one (1) control fish were sacrificed (after being anaesthesized as previously described) at each of the following intervals after injection: 1, 4, 8, 12 and 24 h. The abdominal cavities were then opened; spleens were removed.

**Transmission electron microscopy**

1 $mm^3$ sized first fixed in 2.5% glutaraldehyde in sodium cacodylate buffer at pH 7.2–7.4 for 1 h at room temperature, then for 8 h at 4°C. Splenic tissues were post fixed in 1% osmium tetroxide for 1 h and dehydrated in graded series of ethanol. They were transferred via propylene oxide into epoxy resin embedding medium to produce medium hardness blocks prior to sectioning in a LKB Ultrotome III® ultra-microtome.

Ultra thin sections were stained with uranyl acetate and lead citrate (Chiu et al., 1993). Stained sections were observed with a Jeol-Jem 100S Transmission electron microscope.

## RESULTS AND DISCUSSION

Immunology is the study of the complex cellular and chemical reactions that occur in a host animal in response to contact with a foreign entity, such as a pathogen. An immune response involves recognition of the pathogen by the host as foreign and the subsequent immune response to eliminate it (Roitt, 1998). In recent years, there has been growing concern about the effect of environmental pollutants on the immune system. It is known that phagocytosis is the principal function of macrophages in teleosts. The non-specific immunity in fish**,** similarly to those in higher vertebrates, mainly depends on the activity of monocytes/macrophages, melano-macrophagic centres, neutral granulocytes and thrombocytes (Adeyemo et al., 2002).

In this study, histological sections post injection showed that carbon particles progressively accumulated in the spleen. In the experimental fish sacrificed after 4 h post injection, carbon particles were diffusely distributed in the cytoplasm (Figures 1 and 2), but they gradually form discrete electron dense bodies after 12 h (Figures 3 and 4) that could fuse with some cytoplasmic granules (lysosomes) to form phagolysosomes 24 h post injection of the carbon suspension (Figures 5 and 6). This is in accordance with the study conducted by Esteban and

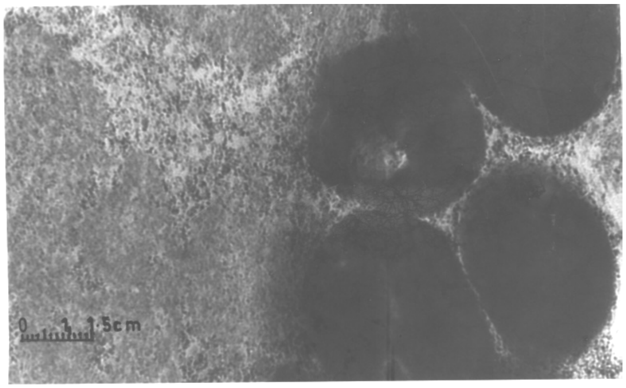

**Figure 4.** High power electron micrograph of fully formed electron dense bodies of aggregated carbon particles. Note that carbon particles are not uniformly distributed in each electron dense body. X 30000 (Let X 2000= 0.1cm, Scale bar = 1.5cm).

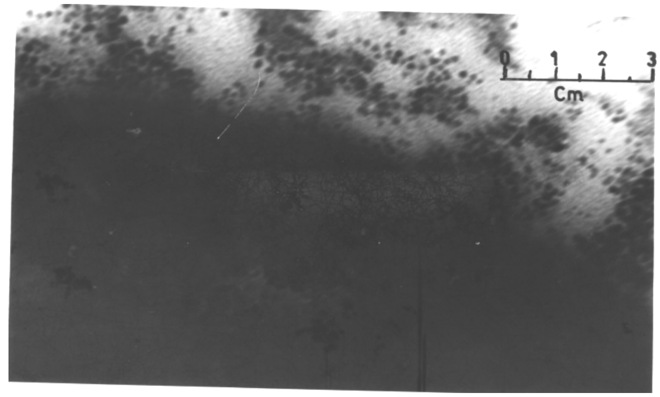


**Figure 6.** High power electron micrograph of carbon particles concentrated in a phagolysosome. X 60000 (Let X 2000= 0.1cm, Scale bar = 3cm).

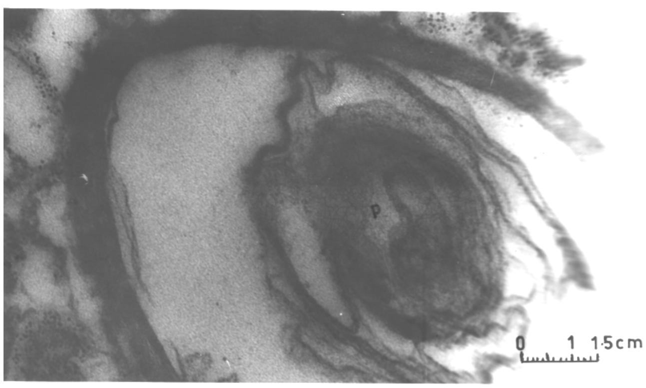


**Figure 5.** Electron micrograph of a phagolysosome (P), which resulted from the fusion of a carbon-laden macrophage with a lysosome. X 30000 (Let X 2000= 0.1cm, Scale bar = 1.5cm).

Meseguer (1997) and Esteban et al. (1998), which indicate that fish monocyte-macrophages may be considered the most active phagocytic cell type. The phagocytic process of *O. niloticus* splenic leucocytes showed similar ultrastructural features to those described for sea bass (*(Dicentrarchus labrax* L.) head-kidney leucocytes (Esteban and Meseguer, 1997) and gilthead seabream (*Sparus aurata* L.) head-kidney leucocytes (Esteban et al., 1998). Macrophages and neutrophilic granulocytes were the two cell types with significant phagocytic capacity in sea bass, which is similar to what has been previously described for rainbow trout (Afonso et al., 1997, 1998). Granulocytes and thrombocytes have also been described as phagocytic cells in some fish species (Rowley et al., 1988). The results reported in this study demonstrate that macrophages and granulocytes are the leucocytes responsible for phagocytic activities in the spleen of this tilapia, in agreement with results from other fish species (Pedrera et al., 1992; Bandin et al., 1993; Lamas and Ellis, 1994).

Previous studies of fish toxicology have targeted ecological parameters such as population dynamics. More recently; however, attention has focused on toxicological pathways and other parameters at the cellular or tissue level (Wester et al., 1994). Toxic effects on the immune system (immunotoxicity) may serve as an excellent tool and as a biomarker to monitor pollution and water quality since most environmental pollutants are known to alter immune system function even at doses that do not produce organ toxicity. Thus, fish immunotoxicology may offer new tools to assess environmental water quality.

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