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# Antigen trapping in the reticuloendothelial system of "*Schizothorax niger* Haeckel"

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**Abstract**
By injecting the colloidal carbon particles, the mononuclear phagocyte system in *Schizothorax niger* was studied *in vivo*. As per results the major organs of carbon particle localization in *Schizothorax niger* after studying histology of organs from 2 hours to day 8 were kidney, spleen, thymus and heart. Spleen was seen with highest density of carbon particles followed by the kidney. The medulla of the thymus was seen first with antigen trapping ability followed by the cortex after day 4. The ventricular endocardial cells of heart were seen as Immunocompetent. Uptake of carbon particles by the monocytes was seen upto day 2.

**Keywords:** colloidal carbon, phagocyte system, kidney, spleen, heart

## Introduction
Mononuclear phagocyte system (MPS) is the widely distributed tissue bound phagocyte system, which is responsible for the phagocytosis. In fish it is the most non-specific defence system during a parasitic attack (Ferguson and Claxton, 1982) [13]. In teleosts the monocytes of the blood stream, mesangial cells of kidney, sinus macrophages of spleen, pro-monocytes of haemopoietic organs, fixed macrophages of atrium of heart and wandering macrophages of loose connective tissue comprise the dispersed and well-coordinated mononuclear phagocyte system of defence. (Ellis *et al.* 1976; Mori 1980; Dalmo *et al.* 1997) [9, 16, 5]. Epidermis and intestine are also the constituents of MPS (Dalmo 1997). The present study was aimed to find out the route taken by the antigen, antigen trapping cells and organs and clearing of the antigen in the fish and to correlate the results with other results and to find out the differences in their mononuclear phagocyte system, if any.

## Materials and Methods
### Experimental Design
Eight healthy *Schizothorax niger* were brought from the fisherman colony near Dal Lake and were maintained in circular fiber tanks at Shuhama Alusteng, Sheri Kashmir University of Agricultural Science and Technology, Srinagar. The water was changed daily and there was a continuous check for temperature, PH, Dissolved oxygen and Algal bloom formation. The temp., PH., and dissolved oxygen were $25^{\pm}$ 1, 7.5 and 6 PPM respectively. They were fed with a formulated pellet diet @ 2.5% body weight twice daily.

### Intraperitoneal injection of colloidal carbon particles
0.5 ml mixture of Indian ink (calmin limited) and normal physiological saline in the ratio of 1:8 was given intraperitonealy to all the fishes. All the eight fishes were sacrificed at the intervals of 2, 8, 16, 48 hours and 4 and 8 days, after the injection.

### Histological studies
The immunocompetent organs (spleen, thymus, kidney, heart etc.) were dissected out for paraffin embedding. Tissue samples were fixed in 10% buffered formalin for 24 hours at ambient temperature (24-28 °C) washed twice with double distilled water and then stored in 70% ethanol. Dehydration, immersion and embedding was followed as done by Humason (1970). 5µms thick sections were cut using a rotatory microtome and then deparaffinized.

For decolorizing the Melanin pigment, the sections were bleached in 20% hydrogen peroxide (Ellis *et al.* 1976) [9]. The sections were stained with eosin and hematoxylin, observed and photographed under a light microscope equipped with a digital camera.

## Results

### Kidney

Two hours after intraperitoneal injection of colloidal carbon, the kidney macrophages were seen with colloidal carbon. This number increased after 8 hours injection and after 48 hours the carbon containing cells continued to aggregate and localize in the haemopoietic tissues. Histology revealed that macrophage cytoplasm was filled with yellow-brick phagocytized debris. The number of aggregates was still increasing and the hematopoietic cells, macrophages, granular leukocytes and lymphocytes were seen in increasing number suggesting that kidney is the main immunocompetent organ in the *Schizothorax niger* as in other teleost fishes.

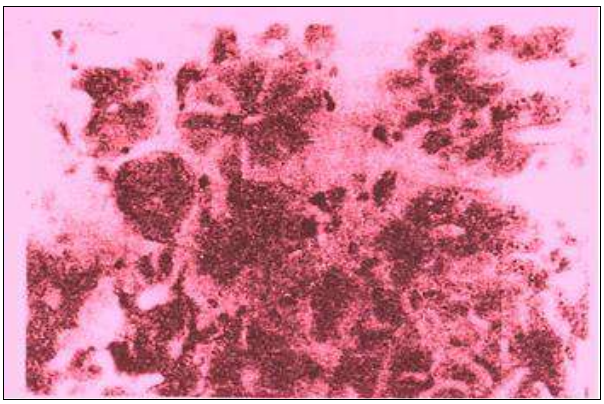

**Fig 1:** Kidney section with carbon particles in macrophages of interstitial tissues after 48 hrs.

### Spleen

After two hours of antigenic challenge the spleen ellipsoids were seen with the carbon particles and the number increased up to 8 hours and the carbon particles were seen in the ellipsoids sheath. The macrophages and other leukocytes were evident. The cells were seen migrating towards the parenchyma after 48 hours of intraperitoneal injection and the number of carbon particle aggregates was increasing up to day 4.On day 8 Melanomacrophage centers were seen in splenic sections and their distribution was highly correlated with the increasing number of colloidal carbon particles. The morphological characteristics of Melanomacrophage centers of spleen samples were similar with the kidney Melanomacrophage centers.

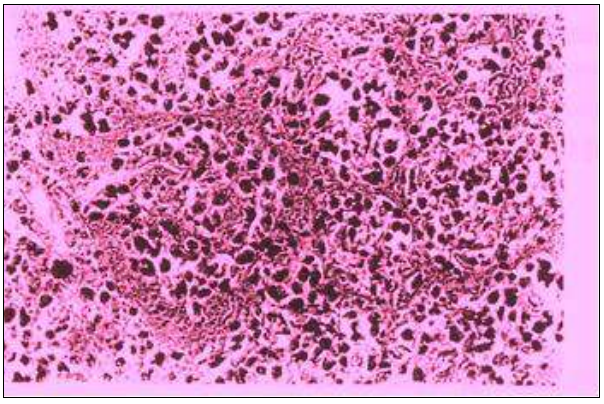

**Fig 2:** Section of Spleen at 24 hrs. with ingested carbon in ellipsoids

### Thymus

It is a pink lobed structure located near the opercular cavity and is also one of the immunocompetent organs in the Schizothorax niger and other fishes. After 2 hours of colloidal carbon particle injection, the medulla was seen trapping the carbon particles as an antigen. After 8 hours of injection of carbon particles the aggregates of carbon particles were seen in the edge of the medulla region and it increased upto day four. After that cortex boundary was also seen with clusters of carbon particles. The thymic parenchyma was seen with the most of the immunocompetence of the thymus in the *Schizothorax niger*, as it occupies much of the Melanomacrophage centers (confirmed by trapping of most of the carbon particles). There was also an increase in the umber of leukocytes and macrophages (wandering macrophages).

### Heart

The heart was also seen as an immunocompetent organ by trapping the colloidal carbon particles with the increasing order from the epicardial lining to the endocardial lining. With just two hours of injection of the carbon particles the epicardium was involved and after 8 hours of injection endocardium was involved in the process of trapping. After 16 hours the ventricular endocardial lining was seen with the carbon particles. This process of increasing carbon particle concentration in the heart continued upto day four, after which there was a mild reduction of the carbon particles.

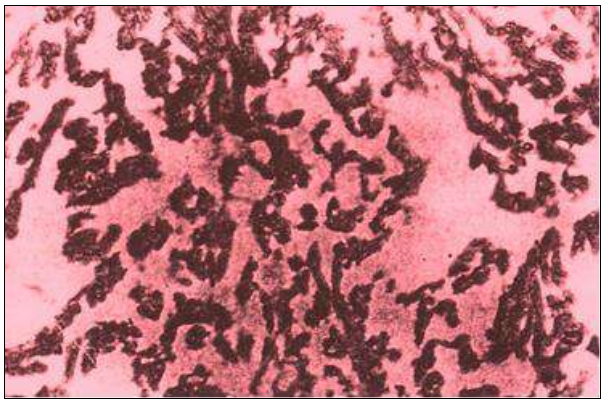

**Fig 3:** Endocardial cells of heart showing carbon particles after 6 days.

## Discussion

As far as the immunocompetent organs of the fishes are concerned the thymus, spleen, kidney and atrium of heart are being considered as the major part players. Same was proved by our experiment conducted on a local fish, *Schizothorax niger*. The Melanomacrophage centers have a role to play in the defence system of the fish. Same was proved by our experiment, where macrophage centers and size correlate with the introduction and timing of antigen. In the bony fish the antigens are trapped in spleen, blood vessels, phagocytic cells of kidney, atrium of heart, thymus.

After injection of the colloidal carbon particles, which are being picked up by the blood phagocytes and transported to various immunocompetent tissues as proved by blood smears prepared after 2 hours of injection, upto that time no organ was seen with colloidal carbon. Subsequently there was an increase of the carbon particle concentration in the spleen (maximum carbon content; Ellis *et al.* 1976) [9].

The role of macrophages in the immune system of *Schizothorax niger* is suggested by the sudden appearance of macrophage centers in the immunocompetent organs (Agius 2004; The increase in white blood corpuscles also suggested their role in the defence system of *Schizothorax niger.*

The role of kidney in trapping of the antigen was proved when colloidal carbon particles were seen just after 2 hours of injection. The endothelial cells were seen with the carbon particles suggesting their role in the phagocytosis (Danneveig *et al.* 1990) [6] After the introduction of colloidal carbon particles in *Schizothorax niger* the macrophages were seen with the debris, that proved our experiment that the macrophages are involved in the phagocytosis (Mori, M.1980) [16] The role played by the granular leukocytes, macrophages lymphocytes and other leukocytes was suggested by their sudden increase in their no. and trapping of the carbon aggregates by the renal portal system, which may be acting as a filter apparatus to trap the blood borne phagocytes containing carbon; Ellis, A.E; Munroe A.L and R.J. Roberts 1976) [11]. Carbon was also trapped by the thymus especially by the Melanomacrophage centers (Sailendri, *et al.* 1975; which are located in the medulla and the cortex boundary (thymocyte maturation center). Several lines of evidences are consistent with the immunocompetent role played by the thymic parenchyma, which was seen as lodging most of the immunocompetence of the thymus of the *Schizothorax niger*. The increase in the number of the leukocytes and tissue macrophages also suggested their role in the defense (Moori M 1980) [16].

Spleen played the major role in trapping of antigen in the present study, where the carbon particles endocytosed cells were seen approaching to the Melanomacrophage centers through parenchymal tissues; Ferguson 1976) [12]. Our results were confirmed by the reports given by Ellis *et al.* 1976 [11] and Ferguson *et al.* 1982 [13]. Splenic ellipsoids are the sites of phagocytosis and diffusion sites for carbon particles in to the parenchyma.

The role of heart in trapping of the antigen was also confirmed by the localization of the trapped carbon particles after 48 hours in the endocardial cells of atrium as also reported by; Ellis *et al.* 1976; Mori 1980) [11, 16]. After atrium the endocardial cells were also seen with the carbon particles after day four suggesting that heart is also an immunocompetent organ of *Schizothorax niger*. Similar results were got by Schimozawa 2001.

Thus, the mononuclear phagocyte system of defence (the nonspecific mechanism of defence) is very important to the fish, though significant differences still exist between various fish species. Thus, the need of the hour is to investigate the major organs of defense in different fishes.

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