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Effect of Diazinon on Marcophages of Bluegill Sunfish, *Lepomis macrochirus:* A Cytochemical Evaluation

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Live specimens of bluegill, Lepomis macrochirus, were exposed to (15 µg L^{-1}, 30 µg L^{-1}, 45 µg L^{-1}, 60 µg L^{-1}, or 75 µg L^{-1}) diazinon to assess the positive PerI's reaction and changes in the microphage dimension in kidneys and spleen. A slight positive PerI's reaction in the renal macrophages of the fish exposed to 15 µg L^{-1} and 30 µg L^{-1} was observed. Larger macrophages and intense positive PerI's reaction occurred with the 45 µg L^{-1} and 60 µg L^{-1} exposures. Macrophage dimension greatly increased at the 75 µg L^{-1} exposure and the positive PerI's reaction became intense. Splenic macrophages exhibited an ascending trend of reaction in their density, dimension and intensity of positive PerI's reaction in the fish exposed to various concentrations (15 µg L^{-1} - 60 µg L^{-1}) of diazinon. Maximum dimension and level of activity were recorded at 60 µg L^{-1}, but their dimension and level decreased at 75 µg L^{-1}. The t-test results exhibit that there are significant differences between the control and the exposed variables in the kidneys and the spleen.

Macrophages are a group of fully differentiated phagocytic cells developed from the immature monocytes and are located in almost every organ system in living creatures. Some researchers investigated the macrophages within connective tissues of kidneys, spleen (Agius, 1985; Munshi et al., 1990; Herraez and Zapata, 1991), liver, gonads (Ellis, 1981), thymus (Gorgollon, 1983), and intestinal lamina (Kambarage et al., 1995). Ziegenfuss and Wolke (1991) studied the macrophage migration pattern in the liver, spleen and kidney of Carrasius auratus using sequential intraperitoneal injection of fluorescent green and yellow micropheres. Pulsford and Matthews (1991) studied the phagocytic activity of macrophages of 17-30 µm in diameter in the muscle tissue of Norway trout injected with a microsporidian parasite. The fish cranial-kidney is considered to be the main macrophage producing tissue (Braun-Nesje et al., 1981). The presence of several types of macrophages, most probably forming part of the same cell lineage, was reported (Braun-Nesje et al., 1982; Hightower et al., 1984; Mesegueir et al., 1991). Macrophages are the principal phagocytic cells in fish (Blazer, 1991).

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Invasion of pathogens affects adversely the physiological status of the fish which in turn activates the hemopoietic tissues to produce a large number of monocytes, and their migration and transformation into macrophages in the inflamed areas and the peripheral lymphoid excretory organs, such as spleen and kidneys respectively. Therefore, any change in pathological status will affect the density and distribution of macrophages in the fish body. Bluegills are commercially important fishes of freshwaters prone to diazinon pollution. Such polluted ambient water will affect adversely the physiological status of the fish resulting in the increase of microphage population in response to pollution . The purpose of the recent study is to estimate changes in the density (diameter of aggregated) and activation of macrophages in kidneys and spleen of bluegills, Lepomis macrochirus and how differently the macrophages in the kidneys reacted to diazinon compared to the spleen.

MATERIALS AND METHODS

Live specimens of bluegill (Lepomis macrochirus) were obtained from a natural fish hatchery near Twin Lakes, Ohio, USA. Fish (total length--l 2.0-13.5 cm, weight--32-45 g) were acclimatized to laboratory conditions for seven weeks. During this period the fish were fed live guppies and commercial fish foodsticks, Tetra Doro Min, manufactured by Tetra Werke, West Germany.

Groups of ten fish for each exposure concentration were exposed in a fiberglass tank (152 x 55 x 36 cm) to different concentrations (15 µg L^{-1}, 30 µg L^{-1}, 45 µg L^{-1}, 60 µg L^{-1}, and 75 µg L^{-1}) of diazinon for 24 h. A separate group of ten unexposed fish was used as control. The water quality of ambient water (temperature--21 ± 1 °C, pH--7.2, DO_2--6.91 mg L^{-1}, total hardness ($CaCO_3$--125.55 mg L^{-1}, and alkalinity--34.57 mg L^{-1} as $CaCO_3$) was the same for control as well as experimental fish. A 14/10 hr light/dark cycle was maintained by using autocontrol fluorescent lighting throughout the holding and experimental period.

Control and experimental fish were anesthetized in the aqueous solution of MS 222, Sigma Co. in their respective ambient water (100 mg L^{-1}) and carefully dissected to remove their renal and splenic tissues for cytochemical tests. After removal of the tissues, the fish was killed with a heavy dose of MS222. In order to prepare smears from the renal and splenic tissues, a small portion of the kidney head and the spleen were excised and carefully tapped onto the clean microscope slides. The smears were allowed to dry and were subjected to Perl's cytochemical test (Pearse, 1972) to demonstrate variations in the density, activation of macrophages, latter's intensity of reaction of control and experimental fish subjected to various concentrations of diazinon.

The PerI's reaction exhibited Prussian blue granules in the splenic and renal macrophages indicating the presence of iron in ferric (FE^{3+}) form temporarily stored in the macrophages as ferritin and hemosiderin owing to phagocytosis of damaged erythrocytes (Munshi et al., 1990). The intensity of phagocytic activity of the macrophages can be recognized by identifying the amount of Prussian blue precipitate.

The stained smears were studied under a microscope and photomicrographs were taken. The dimensions (diameters) of aggregated macrophages were measured directly on photomicrographs randomly and divided by magnification to find actual diameter of the aggregated macrophages. At-test of dimensions of the macrophages in the kidney and the spleen was run between control and exposed bluegill (Table 1).

RESULTS AND DISCUSSION

The renal and splenic macrophages of control and diazinon exposed fish showed positive PerI's reaction for Prussian blue granules (Figs. 1, 2). In control, both in renal and splenic smears, the population density, dimensions and intensity of reactions of macrophages were not significant (Figs. 1A-2A). A slight positive PerI's reaction in the renal macrophages of fish exposed to 15 µg L^{-1} and 30 µg L^{-1} was identified (Fig. 1B, C). Comparatively larger macrophages with intense PerI's reaction were observed in the renal smears of fish subjected to ambient water with 45 µg L^{-1} diazinon level (Fig. 1 D). The population dimensions of renal macrophages and their intensity of Pert's reaction increased when the diazinon level of ambient water was enhanced to 60 and 75 µg L^{-1} (Figs. 1 E, F). But dimension and reaction intensity seem to be more in the 75 µg L^{-1} compared to other exposed fish.

Splenic macrophages showed gradual increases in their population density, dimensions of the aggregation and intensity of reaction in all experimental fish exposed to various concentrations (15-75 µg L^{-1}) of diazinon in their ambient water. There was a significant increase in population density, dimensions of aggregation and intensity of Pert's reaction in splenic macrophages of fish exposed to 15 and 30 µg L^{-1} diazinon (Fig. 2B, C). The maximum level of activity was recorded at 60 µg L^{-1} diazinon concentration (Fig. 2E). However, their level of activity decreased in fish exposed to higher concentrations (75 µg L^{-1}) of diazinon (Fig. 2F). T-test results for both the kidneys and the spleen indicate that there are significant differences between the control and the different concentration variables. In all cases t-values are over two, and 2-tail probabilities are at the highly significant level.

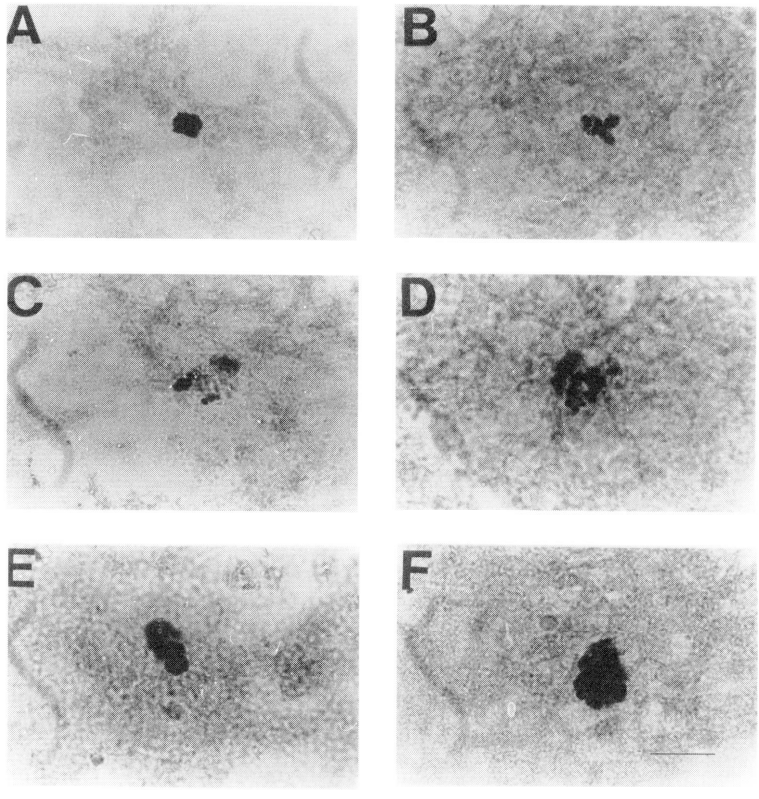

Figure 1. Normal and diazinon exposed kidney.
A, normal kidney; B, kidney of fish exposed to 15 µg L^{-1}; C, kidney of fish exposed to 30 µg L^{-1}; D, kidney of fish exposed to 45 µg L^{-1}; E, kidney of fish exposed to 60 µg L^{-1}; F, kidney of fish exposed to 75 µg L^{-1}. Scale bar = 2 cm, X400.

Although kidneys are excretory organs and the spleen is a lymphatic organ, both function as filtering systems of the blood stream. The fish head-kidney is considered to be the main source of macrophages (Braun-Nesje et al., 1981) and spleen (hemopoietic tissue) possesses many active macrophages (Jungueira et al., 1995).

Exposure of fish to diazinon polluted water causes pathological changes in fish body to activate the monocyte-microphage transformation system of the hemopoietic tissues. In response to intensity of pathological damage the hemopoietic stem cells are activated to form promonocytes which mature to monocytes. The nonphagocytic monocytes are transmitted to various tissues to be transformed into macrophages. The circulating monocytes are

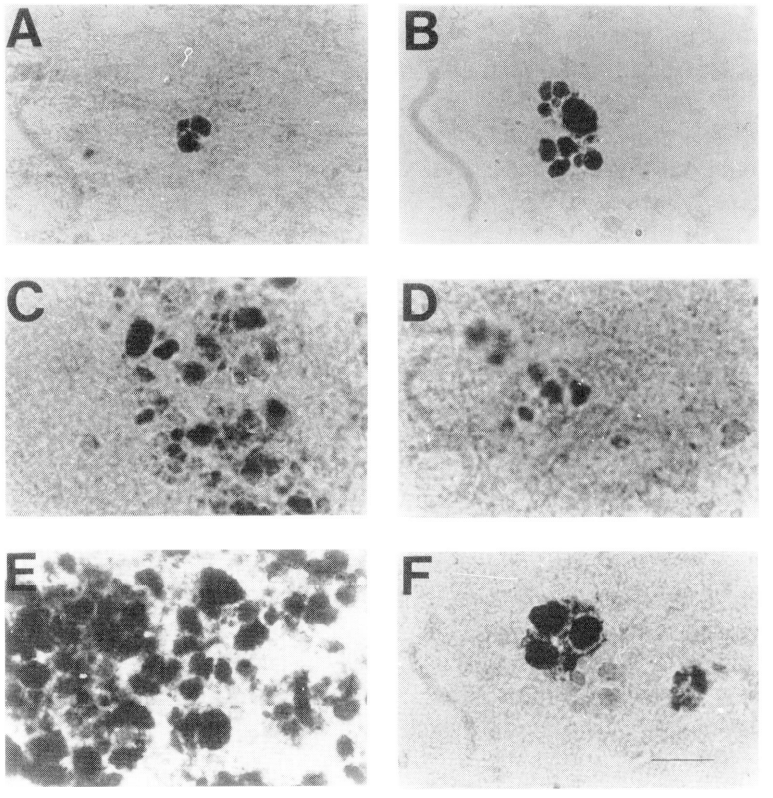

Figure 2. Normal and diazinon exposed spleen.
A, normal spleen; B, spleen of fish exposed to 15 µg L^{-1}; C, spleen of fish exposed to 30 µg L^{-1}; D, spleen of fish exposed to 45 µg L^{-1}; E, spleen of fish exposed to 60 µg L^{-1}; F, spleen of fish exposed to 75 µg L^{-1}. Scale bar = 2 cm, X400.

immature forms of tissue-resident macrophages and it may be possible that tissue macrophages acquire morphological and enzymatic features different from those of circulating peripheral blood monocytes (Kambarage et al., 1995). Kodama et al. (1989) demonstrated a significant activation of macrophages after the rainbow trout (Salmo gairdneri) was immunized with Vibrio anguillarum. Low concentration (15 µg L^{-1}) of diazinon causes little pathological damage in the fish body and therefore, only few splenic macrophages come into action to combat the pathogens. Such a low concentration of diazinon in the ambient water seems to be associated with the damage of the erythrocytes. This assumption is based on the observation of the presence of more macrophages and their intense PerI's reaction in the splenic smear (Fig. 2B, C). Such concentration (15 µg L^{-1}) may be a threshold value to cause any apparent change in the pathological state of the fish in question. Since the spleen is the most efficient filtering system of the body, its macrophages are effective enough to fight the low level of pathological damage caused by low concentrations of diazinon in the ambient water. As macrophages in the spleen take care of most of the low

concentrations (15 -30 µg L^{-1}) of diazinon, this may be one of the reasons for the nonsignificant change in the dimension, density and intensity of PerI's reaction in the renal macrophages of the bluegills exposed to low concentrations (15-30 µg L^{-1}) of diazinon. Significant change in the number and activity to higher concentration (45 µg L^{-1}) of diazinon suggests a maximum response level for the renal macrophages. The maximum number and intense PerI's reaction in splenic macrophages are reflected in the highest response for the bluegills, when the fish were exposed to 60 µg L^{-1} diazinon.

With 75 µg L^{-1} exposure, the dimension of the aggregated population and the reaction intensity of the microphage became much less. This reaction can be attributed to the activation of the fish immune system. Macrophages have a secondary line of function which triggers the T-helper to stimulate the B-lymphocytes which are converted into plasma cells. These plasma cells in turn produce an antibody specific to the antigen. The antibody produced by the plasma cell may destroy the diazinon carrying cells (antigens) leading to the reduction in pathogen cells, which maybe the reason for reduction in the microphage population at 75 µg L^{-1}.

Table 1. T-test of the diameter of aggregated macrophases between the control and diazinon exposed fish at different concentrations. (Number of cases = 10)

KIDNEY				
Levels of Exposure	Mean	SD	T-value	2-Tail Probability
Control	20.02µ	3.13µ		
15 µg L^{-1}	24.75µ	6.53µ	-2.50	.034
30 µg L^{-1}	42.50µ	8.49µ	-8.94	.000
45 µg L^{-1}	48.75µ	5.30µ	-15.92	.000
60 µg L^{-1}	38.00µ	11.54µ	-4.50	.001
75 µg L^{-1}	48.00µ	9.63µ	-8.96	.000

SPLEEN				
Levels of Exposure	Mean	SD	T-value	2-Tail Probability
Control	27.95µ	2.98µ		
15 µg L^{-1}	68.25µ	8.82µ	-14.66	.000
30 µg L^{-1}	158.00µ	47.18µ	-8.70	.000
45 µg L^{-1}	85.00µ	18.18µ	-10.93	.000
60 µg L^{-1}	259.70µ	57.61µ	-12.73	.000
75 µg L^{-1}	63.00µ	5.62µ	-17.39	.000

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