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Acid Phosphatase Activity in the Intestine and Caeca of Bluegill, Exposed to Methyl Mercuric Chloride

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The toxic effects of mercury on fish can be measured accurately by studying the enzymes and other biochemical indicators (Armstrong 1979; Passino 1981; Dutta et al. 1983). The gross organismic changes in fish due to mercury toxicity occur only after significant damage to the internal tissue or physiological-biochemical changes in the tissue. Histochemical and biochemical analyses are reliable procedures for finding out the effects of mercury on the internal tissue before the latter's damage is manifested externally.

Although investigations have been conducted on higher terrestrial vertebrates, few researchers have studied the effects of mercury on enzyme activity in fish (Armstrong 1979; Passino 1981). Armstrong (1979) has provided the basic information on the effects of mercury compounds on fish. Dutta et al. (1983) reported alterations in the blood serum proteins in the bluegill fish exposed to methyl mercuric chloride at a concentration of 3.4×10^{-12} M. Hinton et al. (1973), Hinton and Koenig (1975), Jackim et al. (1970), Manen et al. (1976), Wekell and Brown (1973) made investigations towards the effects of mercury on the enzymes of certain target organs like liver and kidney of fish.

The intestine has contact with the external environment via food, while the gills have direct and more contact with the environment. Therefore, any significant alteration in the environment may induce some changes in the structure and function of the digestive tract. In view of the above, an attempt has been made to measure the changes in acid phosphatase activity in different regions of intestine and intestinal diverticulae of bluegill fish as these enzymes are highly sensitive to methyl mercury.

MATERIALS AND METHODS

A total of 18 bluegill fish (Lepomis macrochirus) 30-45 g weight and 12-15 cm long were obtained from a local reservoir in Kent, Ohio. The fishes were acclimatized in an aquarium of 400 L for one week prior to the experiment. Nine of the 18 fishes were kept as the control in one aquarium while the remaining nine were

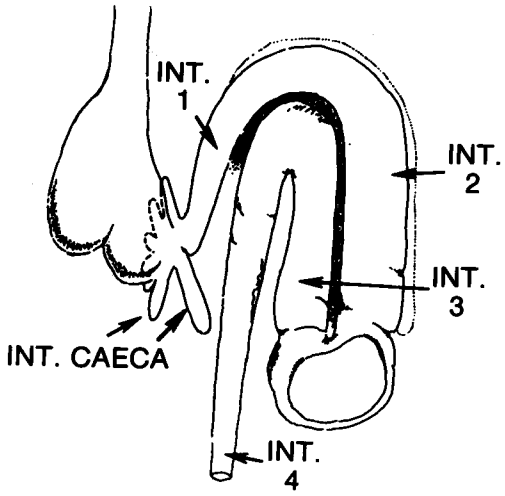

Plate 1. Different regions of intestine and intestinal caeca

exposed to 3.4 X 10^{-12}M methyl mercuric chloride. Both control and experimental fishes were not fed during the experiment. The concentration of methyl mercuric chloride for this study was selected after reviewing both lethal and sub-lethal doses as used and recommended by the previous investigators (Armstrong 1979; Dutta et al. 1983). Two aquaria (61cm x 31cm x 31cm) were used, each having 12 L of static, pH = 7.25 and temperature 22-25^{o}C water.

Each three fishes from both control and exposed were sacrificed for each period of exposure (24 hr, 48 hr, and 72 hr). Acid phosphatase activity was determined using the modified Gomori (1941) metal precipitation technique as described by Bancroft (1975).

Regional investigations on the acid phosphatase distribution were done by taking 10-micron thick sections from four regions (INT_1, INT_2, INT_3, and INT_4) of the intestine, and intestinal caeca (Plate 1). Thin sections were obtained using a cryostat. The optimum incubation medium was maintained by controlling pH and temperature as suggested by Bancroft (1975). In accordance with the Bancroft (1975) procedure controls were run simultaneously to check the correct localization of the enzyme. At least 10 sections from each of the 4 intestinal regions and 2 sections from each of the caeca (Plate 1) were thoroughly examined to qualitatively grade the degree of acid phosphatase activity.

RESULTS AND DISCUSSION

The results show that in both control and mercury exposed bluegill fish the first part of the intestine (INT_1) possesses

Table 1: Acid Phosphatase Activity in the Intestine and Caeca of Bluegill Fish

Regions of Intestine & Caeca	Normal Fish									Methyl-mercury Exposed Fish								
	24 hr			48 hr			72 hr			24 hr			48 hr			72 hr		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
INT 1	A^{2-3}	A^{2}	A^{2}	A^{2}	A^{2}	A^{2}	A^{2}	A^{2}	A^{2-3}	A^{3}	A^{2-3}	A^{3}	A^{3-4}	A^{4}	A^{3-4}	A^{4}	A^{4}	A^{3-4}
INT 2	A^{1}	A^{1-2}	A^{2}	A^{1}	A^{1}	A^{1-2}	A^{1-2}	A^{1}	A^{1-2}	A^{1-2}	A^{2-3}	A^{2}	A^{2-3}	A^{2-3}	A^{3}	A^{3}	A^{2-3}	A^{2-3}
INT 3	A^{1-2}	A^{1}	A^{1-2}	A^{1-2}	A^{1}	A^{1}	A^{1}	A^{1}	A^{1-2}	A^{2}	A^{1-2}	A^{2}	A^{2-3}	A^{2-3}	A^{2}	A^{3}	A^{2-3}	A^{3}
INT 4	A^{1}	A^{1-2}	A^{1}	A	A^{1-2}	A^{1}	A^{1-2}	A^{1}	A^{1}	A^{2-3}	A^{3}	A^{2-3}	A^{3}	A^{3-4}	A^{3-4}	A^{3}	A^{4}	A^{4}
Caeca	A^{1}	A^{0}	A^{1}	A^{1}	A	A	A^{1-2}	A^{1}	A	A^{0}	A^{1}	A	A^{1}	A^{1-2}	A^{1}	A^{1}	A^{1}	A

INT 1, 2, 3 & 4: Intestinal region 1, 2, 3, & 4 respectively

Degree of acid phosphatase activity has been indicated in the following order:

A^{0} - No Activity; A - Low Minimum; A^{1} - Minimum; A^{2} - Low Moderate; A^{3} - Moderate; A^{4} - Maximum

higher enzyme activity (Plate 1, Table 1, Fig. 1a,b) in comparison to other parts of the intestine. The rest of the regions (INT_2, INT_3 and INT_4) of the intestine in control group show variable degree of enzyme activity ranging from low minimum to low moderate while the INT_2 and INT_3 of the intestine of the exposed group showed variable but higher degrees of activity (low moderate to moderate) (Plate 1, Table 1, Fig. 2a,b). Such variations made it difficult to formulate any definite pattern of phosphotase activity in the intestine of bluegill fish.

In both groups the intestinal caeca showed lower activity in comparison to that of the intestine. The caeca in the exposed fish did not show any clear indication of increased activity with time (Table 1, Fig. 3a,b).

However, when control fish were compared wih the mercury-exposed fish, a common trend of gradual increase in phosphatase activity from 24 hr to 72 hr had been noticed in all parts of the intestine of the exposed fish (Tab. 1). Besides this trend of increased activity in exposed fish, the increase was unusually high in the anal part (INT_4) of the intestine (Fig. 4a,b).

The relatively higher activity of acid phosphatase in the first part (INT_1) and heterogeneity of activity in the remaining parts of the intestine may be explained by the variations in morphology and functions of these different intestinal regions as well as by the different rates in the flow of the food-stuff in the intestine. Although the fish were not fed during the experiment, some previous studies indicate that food can remain for a longer period of time in the digestive tract (Lagler et al. 1977). Therefore, the flow of the food might be playing a vital role because the semidigested food coming down from the stomach is not uniformly distributed throughout the intestine. Hence in addition to structural and functional variations of the different intestinal regions, the random presence of food-stuff in one part and its absence in another part perhaps make random variations in the phosphatase activity in the intestine. The consistent higher enzyme activity in the first part (INT_1) occurs possibly due to its direct attachment to the stomach where food is stored and may also be related to its higher secretory function than that of other parts of the intestine. Sastry (1974) and Stroband (1980) reported distinct regional differences in the distribution pattern of certain enzymes in the normal fish intestine. Sastry (1974) showed that the anterior portion of intestine is consistently more rich in esterase and lipase activiy than in the posterior portion. Stroband's (1980) study on the intestine of grass carp shows a regional pattern with a proximal-distal gradient in alkaline phosphatase activity. Therefore, the occurrance of comparatively higher enzyme activity in the proximal portion of bluegill intestine confirms the findings of both Sastry (1974) and Stroband (1980).

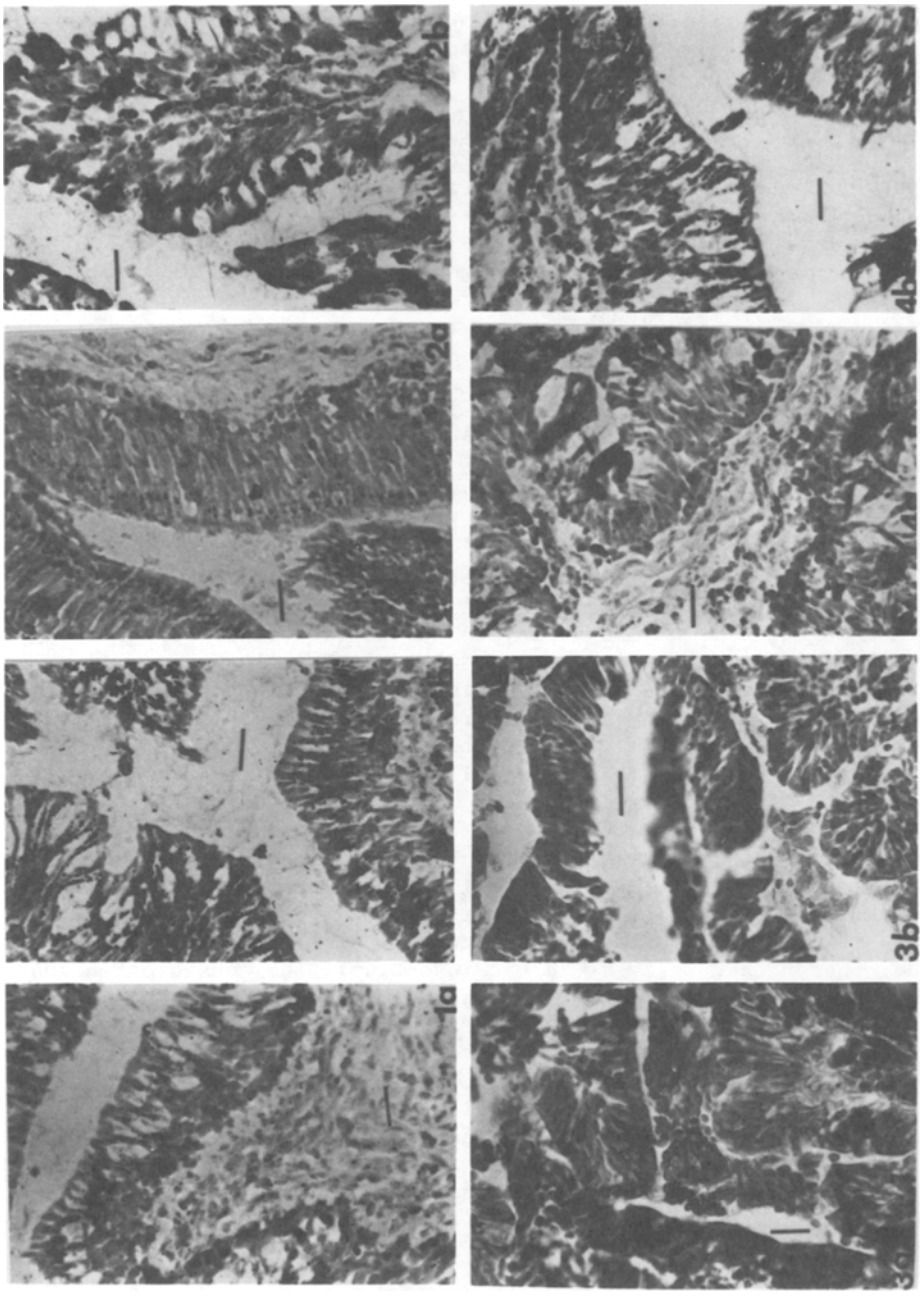
2b
4b
2a
3b
1a
3a

Figure 1a,b: 1st part of intestine (INT_1)
a) Control, showing high activity (low moderate to moderate) magnification X350, bar = 25 µm
b) 48 hr mercury-exposed fish, showing increasing activity (moderate to maximum), magnification X350, bar = 25 µm

Figure 2a,b: 2nd part of intestine (INT_2)
a) Control, showing low activity (minimum to low moderate), magnification X350, bar = 25 µm
b) 72 hr mercury exposed fish, showing increasing activity (low moderate to moderate), magnification X350, bar = 25 µm

Figure 3a,b: Intestinal caecum
a) Control, showing very low activity (no activity to low moderate), magnification X350, bar = 25 µm
b) 48 hr mercury exposed fish, showing no significant trend of increased activity (minimum to low moderate), magnification X350, bar = 25 µm

Figure 4a,b: Anal part of intestine (INT_4)
a) Control, showing low activity (low minimum to low moderate), magnification X350, bar = 25 µm
b) 72 hr mercury exposed fish, showing increasing activity (moderate to maximum), magnification X350, bar = 25 µm

High activity in the anal part (INT_4) of the exposed fish might have resulted from direct contact of the anal region with the mercury contaminated water.

Low phosphatase activity in the caeca probably indicates that though caeca increase digestive surface area (Legler et al. 1977; Stroband 1980) the entire food does not pass through them, hence the production of low enzyme in this structure is not unnatural. Some authors (Romer 1970; Reifel and Travill 1979) reported that these intestinal diverticulae function as the breeding places and reservoirs for the normal intestinal flora. They are also of the opinion that none of these caeca appear to be associated with any strong secretory function. However, among the enzymes present in these diverticulae, the lipase activity is reported to be relatively high (Stroband 1980; Goel 1974; Lagler et al. 1977).

A generally higher acid phosphatase activity in all parts of the intestine in mercury-exposed fish indicates that mercury at sub-lethal dose stimulates enzyme activity. The induction of acid phosphatase seems to be needed for the conversion of the toxic material into less toxic product (Armstong 1979; Doull et al. 1980; Hinton et al. 1973; Passino, 1981). Hinton and Koenig (1975) found inhibition of acid phosphatase at 15 mg Hg/kg concentration, but at 1.5 mg Hg/kg there was an increase in

activity. Jackim et al. (1970), who measured the effect of mercuric ion upon liver enzymes in Killi fish, showed an increase in alkaline and acid phosphatase at concentrations of 0.23-0.17 mg Hg/L but inhibition was noticed at a higher concentration. Therefore, on the basis of previous reports and the results obtained in this study, it appears that both induction and inhibition of phosphatase may take place depending on the concentration of the mercury. Thus, it has been postulated that in the exposed fish an acute toxicity at lethal dose causes phosphatase inhibition whereas chronic toxicity at sub-lethal dose causes phosphatase activation.

Hinton et al. (1973) reported that mercury in fish tissue causes enzyme alteration even at very low concentrations. Upon continued exposure toxicity leads finally to structural alterations. In this study no structural changes were found at this low sub-lethal level of methyl mercuric chloride and in the short period of exposure. The absence of tissue damage in this study and its presence in Hinton's is probably due to the extended exposure time of his experiment.

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