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Parathion and Salinity Effects on Gills and Mesonephros Carbonic Anhydrase Activity of the Fish *Oreochromis hornorum*

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Pesticides are an important kind of the water contaminant because they are potentially toxic to fish, eliciting biochemical changes and, consequently, effects on the survival, growth, and reproduction (Haider and Upadhyaya 1986). Parathion, as well as other organophosphate insecticides, inhibit acetylcholinesterase with subsequent disruption of nervous function (Reddy and Rao 1988). Pant and Singh (1983) found that organophosphorus compounds, at sublethal concentrations, affect carbohydrate and cholesterol metabolism in the fish *Puntius conchonius*. Salinity is another environmental factor affecting fish biochemical parameters (Dharmamba *et al* 1975; Ahokas and Duerr 1975), as well as ion fluxes and transepithelial electrical potential (Dharmamba *et al* 1975; Young *et al* 1988).

Gill and mesonephros serve acid-base and osmoregulatory functions, which also involve carbonic anhydrase action, because this enzyme catalyzes the interconversion between CO_2 and carbonic acid or some of its ionic forms, therefore it should be involved in the acid-base balance. Swenson (1984) found that gill carbonic anhydrase in the shark is involved with both CO_2 exchange and HCO_3^- excretion. Kerstetter and Kirschner (1974) found in trout a HCO_3^--dependent ATPase activity. In this work the effects of parathion and salinity were tested on the carbonic anhydrase activity in both gill and mesonephros of the fish *Oreochromis hornorum*.

MATERIALS AND METHODS.

Young fishes *Oreochromis hornorum* weighing between 5 and 9 g, caught in the freshwater Piscicola Center of Zacatepec Morelos, México, were carried to laboratory

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and, in order to avoid any infection by ectoparasite, were kept 3 d in a 0.0005% methylene blue solution. Then, the fish were kept 7 d in the laboratory conditions for its adaptation. The animals were fed daily with groundnut cake. Tap water at 20±2 °C was used, which was bubbled with oxygen continuously.

The LC_{50} (0.417 mg/L) was previously determinated in our laboratory by using a dilution technique, with six groups of 10 fish each. Five groups were exposed for 72 hr to 114, 154, 191, 239 or 342 mg/L of parathion. The last group, maintained in parathion-free solution, served as the control.

When sublethal parathion concentrations were used, the fish were distributed into ten groups, six per group, in 17-L aquarium jars. Four groups were exposed, for 72 hr to sublethal parathion concentrations: 0.0083, 0.0104, 0.0139, 0.0208 mg/L, which correspond to 1/50, 1/40, 1/30 and 1/20 of the LC_{50}, respectively. Laboratory tap water was used, which had 1.1% of salinity (low salinity). pH 7.2 - 7.5, alkalinity (as $CaCO_3$) 95-110 mg/L, hardness (as $CaCO_3$) 118-122 mg/L at 20°C. Other four groups were exposed at the same parathion concentrations, but NaCl was added to get a salinity of 3.1 % (high salinity). The other two groups were for control, one for low and the other one for high salinity without parathion. The exposure to parathion was carried out at room temperature (19-22 °C). After the intoxication period, fish were weighed and killed by decapitation; then, gills and mesonephros were isolated. These organs were homogenized in ice-cold deionized water (1.0 g/mL of fresh tissue).

Carbonic anhydrase activity of gills or mesonephros was determinated by Maren's micromethod (1960), which consists in measuring the time of the CO_2 hydration reaction in a mixture of phenol red, a sample containing the supernatant of the tissue homogenized and a Na_2CO_3-$NaHCO_3$ buffer. The reaction time (RT) was taken between the moment of buffer addition and that when the mixture turned from red to yellow. All procedures were carried out at 4-5 °C. The enzyme unit was taken as that amount of carbonic anhydrase able to diminish the RT to 50 % of that for the uncatalyzed reaction.

Each point in the graphics is the average of three experiments, taken five measurements per experiment (n=15). ANOVA two ways (salinity and parathion concentration) was used, and Duncan's test for multiple comparison between means, except for differences between gills and mesonephros without parathion since they were treated as independent samples and therefore

two-tails Student's "t" test was used (28 degrees of freedom). Values of carbonic anhydrase activity given in the text are mean ± standard error of the mean.

RESULTS AND DISCUSSION

ANOVA test showed significant effects on carbonic anhydrase activity elicited by parathion concentration and by salinity, in gill as well as in mesonephros, besides interaction between both factors ($P<0.05$).

Carbonic anhydrase activity in low salinity, without parathion, resulted 687±21 and 1087±175 EU/g of fresh tissue of gills (Fig.1) and mesonephros (Fig. 2), respectively. Thus, the activity was significantly greater in mesonephros than in gills homogenized ($P < 0.05$). With high salinity the activity in gills was increased to 1195±95 EU/g, which is 74 % greater than the control at low salinity (Fig.1) ($P<< 0.05$), but in mesonephros the activity was 1430±177 EU/g which is not statistically different of its control (Fig.2)

When parathion, at nominal sublethal concentrations, was used in low salinity, the gills carbonic anhydrase activity was not affected at the two lower parathion concentrations (1/50 and 1/40 LC_{50}), but at the two higher concentrations (1/30 and 1/20 LC_{50}). The activity significantly increased ($P<0.05$), from 687±21 EU/g in control to 1198±9 EU/g at the highest parathion concentration , which represents an increase of 74 % with respect to control without parathion. In high salinity, with parathion concentration of 1/50, 1/40 d 1/30 LC_{50}, the carbonic anhydrase activity decreased with the parathion concentration getting a bottom of 670±8.3 EU/g at 1/30 LC_{50}, which is statistically equal to that observed without parathion in low salinity. However, at the highest concentration (1/20 LC_{50}), carbonic anhydrase activity resulted 2473±180 EU/g which represent an activity augment of 100 % the control at high salinity ($P<<0.01$), (Fig.1).

In mesonephros at low salinity, the lowest parathion concentration (1/50 LC_{50}) did not produce any significant effect on carbonic anhydrase activity, but at the next parathion concentration the activity fell from 1087±21 EU/g (control) to 469±76 EU/g, which is significant ($P < 0.05$). At the highest parathion concentration (1/20 LC_{50}), the activity increased to values (1083±177 EU/g) near the control. When the fish were kept in high salinity, at the lowest parathion concentration (1/50 LC_{50}), the mesonephros carbonic anhydrase activity increased to 2680±308 EU/g (87 % greater than control at high salinity). However, at the next concentration (1/40 LC_{50}) the activity fell at

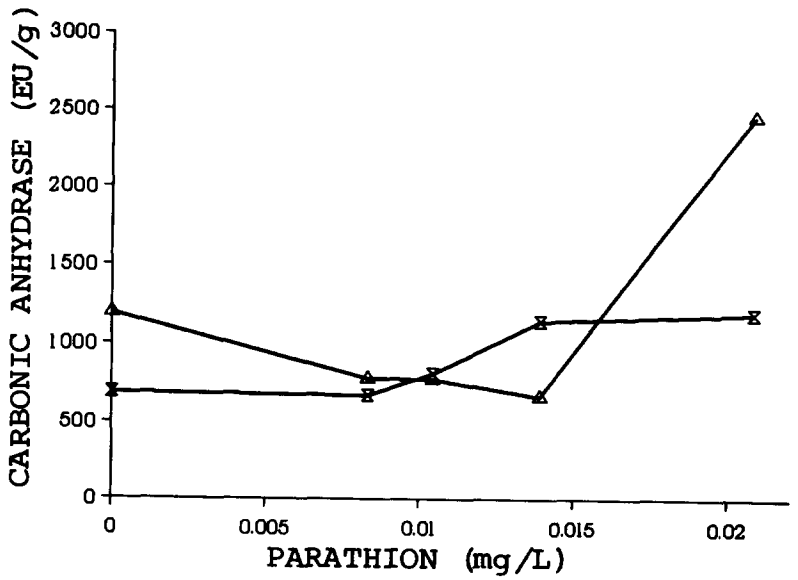

Figure 1. Parathion and salinity effects on carbonic anhydrase in gills homogenized of the fish *Oreochromis hornorum*. (x) low salinity, (Δ) high salinity.

697±10 EU/g, and at the two higher concentrations (1/30 and 1/20 LC_{50}), it arose to a value (1309±8.2 EU/g) none statistically different from those observed in control (low or high salinity). These results are shown in Figure 2.

The remarkable facts about the above results are: a) In low salinity without parathion, the carbonic anhydrase activity in gills was lower than in mesonephros, but in high salinity the activity in gills increased, whereas in mesonephros it was not changed. b) In mesonephros with high salinity, the lowest parathion concentration elicited a strong activity increase, whereas at low salinity, at the same parathion concentration, no effect was observed, but at the next concentration the activity in both salinities was diminished and then a tendency to recover the control activity was observed. c) In gills, a tendency to recover the control values was not observed, neither in low nor high salinity, although in both cases the carbonic anhydrase activity was higher than the control values, but very much greater in high than in low salinity. This last result, suggests a potentiation of the parathion effect on gills carbonic anhydrase activity in high salinity, which could be related to the statistical interaction between both factors.

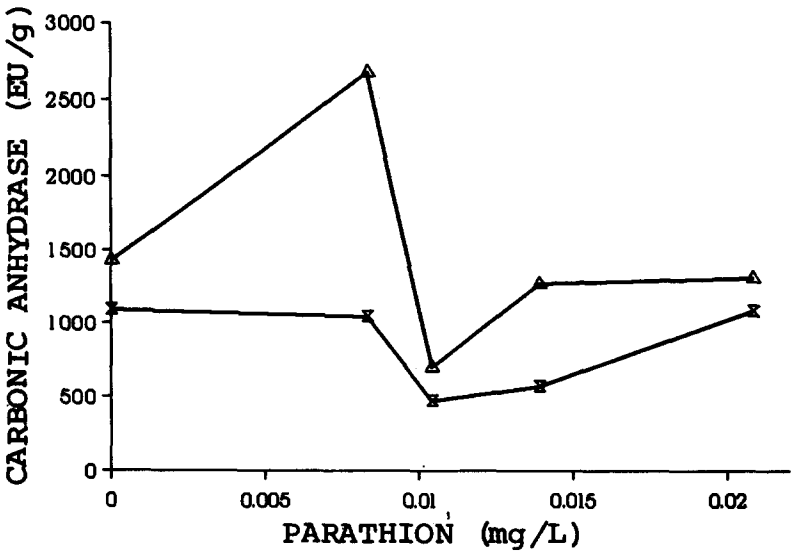

Figure 2. Parathion and salinity effects on mesonephros carbonic anhydrase activity of the fish *Oreochromis hornorum*. (x) low salinity, (Δ) high salinity.

An ample variety of epithelial cells, as well as erythrocytes and other tissues, can exchange bicarbonate for chloride across the cellular membranes. The gill of the fish posseses a HCO_3^--dependent ATPase which may be involved in the Cl^-/HCO_3^- exchange. This mechanism seems to be modulated by carbonic anhydrase, catalyzing the interconversion of CO_2-HCO_3^-. Therefore, this system should be related to the intracellular pH maintenance. According to these considerations, one may think that when the fish is bathed in a low chloride solution, the cells of the gill do not require a high carbonic anhydrase activity to supply HCO_3^-, to the anion exchange mechanism. However, when chloride concentration is high, this might induce, in some way, an increased carbonic anhydrase activity, in order to supply HCO_3^- more rapidly to the anionic exchanger, until reach a new steady state. Nevertheless, in mesonephros this requirement is not so pressing because the composition of the extracellular fluid may be regulated by several homeostatic mechanisms. The above hypothesis might explain the differences of carbonic anhydrase activity between gills and mesonephros without parathion, at low salinity, as well as the salinity effects on both tissues.

At the present we do not have an explanation about how the parathion acts in changing the carbonic anhydrase

activity, but, since the activity of this enzyme seems to be modulated by cyclic AMP, at least in other tissues (Galar and Marroquin 1991), it is possible this insecticide perturbs the system involved whit the cAMP synthesis. Then, it is reasonable to explore this hypothesis in future trials, including other parameters as ionic fluxes and intracellular and transepithelial electrical potential.

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