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Hematological Responses of Larval *Rana catesbiana* to Sublethal Nitrite Exposures

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Nitrite (NO_2^-) is an intermediate compound formed during nitrification of ammonia which is the major nitrogenous waste product of aquatic animals. When excreted into water ammonia is biologically converted as follows:

Ammonia —(Nitrosomonas)⟶ Nitrite —(Nitrobactor)⟶ Nitrate.

In aquatic culture systems, ponds, laboratory holding systems, sewage plant receiving waters and natural systems where animal biomass is high, nitrite can reach lethal and/or limiting levels particularly if imbalances occur in the relative abundances of Nitrosomonas and Nitrobactor.

In contrast to many toxic chemicals in which the underlying mode of action is neither known nor studied, various investigators (SMITH and WILLIAMS 1974, RUSSO et al. 1974, HUEY et al. in press) have hypothesized that nitrite exerts its toxic action by rapidly converting hemoglobin to methemoglobin (BODANSKY 1952), a form incapable of carrying oxygen. If a large proportion of methemoglobin is present tissue anoxia can develop.

Nitrite induced methemoglobin formation has been reported for various salmonid fishes (BROWN and McLEAY 1975, SMITH and RUSSO 1975), chinook salmon, Oncohynchus tschawytscha, in freshwater and seawater (CRAWFORD and ALLAN 1977) and channel catfish, Ictalurus punctatus (TOMASSO et al., 1979 and HUEY et al., in press). Methemoglobinemia is reduced in the presence of monovalent anions (PERRONE and MEADE 1977, RUSSO and THURSTON 1977, WEDEMEYER and YASUTAKE 1978, TOMASSO et al. 1979, HUEY et al. in press).

With the exception of a single, short paper (SULLIVAN and RIGGS 1964) reporting methemoglobin occurrence in the redear turtle (Chrysemys scripta), an extensive literature search revealed no other reports of methemoglobin in nonfish aquatic vertebrates. For intertaxon comparative purposes, we designed research to determine if bullfrog tadpoles (Rana catesbiana) would develop methemoglobin when exposed to nitrite and if so, whether increases in ambient chloride concentrations would offer protection against methemoglobinemia.

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MATERIALS AND METHODS

Tadpoles (15 g to 26 g) obtained during spring from a local supplier were held in 200 L tanks containing dechlorinated, continuously filtered tapwater at 23-25°C. Prior to and following a 5 day holding period, holding water was analyzed for ammonia and nitrite with calibrated Orion specific ion probes.

Tadpoles were transferred to 25 C, medium hardness, (137 total hardness mgL^{-1}) low chloride, (5.0 mgL^{-1}) pH 7.3 water for static dose-response tests. Chemicals were added to 30 L aerated test tanks and mixed by a mechanical stirrer. After two hours 6 tadpoles were distributed into each test tank. Nitrite levels were monitored at 0, 12, and 24 hr intervals while other water parameters (ammonia, total hardness, chloride, pH, temperature) were monitored at 0 and 24 hr intervals. Chemicals were reagent grade and sodium nitrite was used as the nitrite source.

Tadpoles were exposed to nitrite concentrations (mgL^{-1}) of 50, 10, 5, 3, 1 and control (<.01) in the dose response experiment. Chloride inhibition tests employed 50 and 10 mgL^{-1} NO_2 exposures with molar $Cl:NO_2$ ratios of 13:1 and 5.2:1 respectively.

Following 24 hr exposure, blood was collected from individual animals in heparinized capillary tubes. Total hemoglobin was quantified by the cyanomethemoglobin method (HAINLINE 1958) and blood methemoglobin determined by a method modified from that of EVELYN and MALLOY (1938).

RESULTS AND DISCUSSION

Dose response experiments indicated that although total hemoglobin levels were not significantly altered a strong, positive relationship was found between methemoglobinemia and nitrite concentration over the test range of 1.0 to 50 mgL^{-1} (Table 1).

Table 1. Hematological responses of bullfrog tadpoles following 24 hour exposures to nitrite. For each exposure, sample size equalled 6 and mean ± 1 standard deviation are given.

Exposure	Hemoglobin g 100 ml^{-1}	Methemoglobin g 100 ml^{-1}	Methemoglobin %
Controls	5.4 ± 0.9	0.3 ± 0.1	5.7 ± 5.7
1.0	5.1 ± 1.1	1.1 ± 5.1	21.4 ± 12.9
3.0	6.4 ± 0.7	1.5 ± 0.6	32.1 ± 11.5
5.0	6.4 ± 1.3	2.1 ± 0.4	34.3 ± 9.8
10.0	6.1 ± 0.7	2.6 ± 0.3	43.7 ± 8.6
50.0	5.7 ± 0.6	2.9 ± 0.7	51.0 ± 11.1
10.0[a]	6.1 ± 0.9	0.3 ± 0.1	5.6 ± 0.9
50.0[b]	4.9 ± 1.0	0.1 ± 0.1	2.0 ± 0.7

a and b included addition of chloride at molar $Cl:NO_2$ ratios of 13.1 and 5.2:1 respectively.

Percentage methemoglobin was highly significantly correlated ($p<0.001$) to nitrite concentration in a double logarithmic fashion. The best fit regression model was: $\log_{10}$ MHb (% total Hb) = 1.328 + 0.271 $\log_{10}$ NO_2^- (mgL^{-1}). Standard errors for the intercept and slope equalled 0.0209 and 0.0178, respectively; and the coefficient of determination (i.e. R^2) was 0.98.

The percentage MHb of all exposure groups shown in Table 1 are significantly different from the control group which had a mean of 5.7% MHb (Anova $p<0.001$). These results indicate that tadpoles are more resistant to nitrite induced methemoglobinemia than channel catfish under similar conditions (HUEY et al., in press). Channel catfish exposed to 5.0 mgL^{-1} nitrite developed methemoglobin levels of 90% which are approximately three times those observed in tadpoles (Table 1). The reduced methemoglobin response of tadpoles is related to reduced nitrite uptake and/or the presence of an efficient methemoglobin reductase system.

Tadpoles exposed to 50 mgL^{-1} nitrite at molar ratios of 13:1 ($Cl:NO_2$) and 5.0 mgL^{-1} at 5.2:1 ($Cl:NO_2$) had methemoglobin levels that were not elevated above control levels (Table 1). This suggests that chloride provides protection against methemoglobinemia at much lower levels in tadpoles than channel catfish. The latter species require a 17:1 ($Cl:NO_2$) ratio for full protection (TOMASSO et al. 1979).

Resistance to nitrite toxicity - methemoglobinemia in tadpoles is most likely an uptake phenomenon. Bullfrog tadpoles show gill ion absorption (ALVARADO and KIRSCHNER 1963) similar to fishes. Tadpoles at this stage (late larval) have limited gill surface area when compared to fish and hence a lower dose response would be expected. This could also account for increased efficacy of chlorides as nitrite toxicity protectors.

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