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Influence of Lead, an Environmental Pollutant on Metamorphosis of Rana utricularia (Amphibia: Ranidae)

Gloria L. Yeung
University of Arkansas, Fayetteville

The Influence of Lead, An Environmental Pollutant on Metamorphosis of *Rana utricularia* (Amphibia:Ranidae)

GLORIA L. YEUNG
Department of Zoology
University of Arkansas - Fayetteville
Fayetteville, Arkansas 72701

ABSTRACT

The influence of lead, an environmental pollutant on metamorphosis of *Rana utricularia*, was examined in this study. Larvae of stage XI (Taylor and Kollros, 1946), beginning of the premetamorphic stage, were exposed to lead concentrations of 0.1 ppm, 0.5 ppm, 1.0 ppm and 1.5 ppm for 106 days. The untreated larvae served as controls. Larvae were observed from early limb bud stage, stage X, through the protrusion of the forelimbs, stage XX. Neither the sequence of developmental events nor the gross external and internal morphology was altered by lead. However, lead prolonged the time of metamorphosis by delaying the completion of each successive prometamorphic stage. The extent of delay was concentration dependent. The thyroid gland of the experimental larvae underwent alterations which were also concentration dependent. The size of the gland and the size of the follicle were reduced. Vacuolation occurred in the colloid of both 0.5 ppm and 1.5 ppm lead treated larvae.

INTRODUCTION

Lead is one of the environmental pollutants which has become more prevalent due to mechanization and industrialization. Lead particles enter the atmosphere from leaded gasolines, pesticides, manufacturing, combustion of coal, incineration of refuse, and leaded paints (Pagenkoff and Neuman, 1974). Lead mining industries are another source of environmental contamination. The lethal effect of environmental lead on organisms was first reported by a lead-polluted stream to the presence of the metallic salts dissolved in the water. Investigations concerning metallic pollutants have focused on various invertebrates and vertebrates as indicator organisms for toxicologic study; the toxicity of lead has been demonstrated in both embryonic and adult forms of bivalves (Eisler, 1977), in zooplankton (Baudouin and Scoppa, 1974), in birds (Benson et al., 1974 and Benson et al., 1976) and in tadpoles (Bell, 1924). Sublethal efects of lead also have been documented in man and other vertebrates (for review, see National Academy of Science, 1972), but few investigators have considered long term effects due to lead from environmental sources on developing organisms.

Metamorphosis is a developmental phenomenon frequently associated with a change in habitat by an organism, such as from an aquatic habitat to a terrestrial habitat by frogs and toads. Adaptation to a new environment requires considerable transformation of both structure and function in the living organism (Berrill, 1971). These transformations are considered to be related directly to the activity of the thyroid (Lynn and Wachowski, 1951). Significant alterations of the environment might be expected to complicate developmental processes, such as metamorphosis, and possibly to alter morphological and histological development of the organism.

Since the effect of lead on metamorphosing organisms has not been documented, this experiment was designed to examine the influence of lead on metamorphosing larvae of amphibians. *Rana utricularia* was chosen as the experimental animal because of its availability and relatively short metamorphic period, two to three months. Particular attention was paid to the time required for the completion of the events of each stage of metamorphosis, the sequence of metamorphosis, and normalcy of morphological changes. Finally, since the function of the thyroid may be altered by the action of lead, this possibility was approached indirectly by examining the histology of the thyroid gland.

MATERIALS AND METHODS

The larvae of *Rana utricularia* were collected from a shallow pond near Lake Wedington in Washington County, Arkansas, in early May. The larvae were reared in the laboratory at a density of five organisms per liter of spring water which had a lead concentration of five parts per billion. Water temperature was maintained at 21°C ± 1°C and the water was changed every other day. The larvae were fed cooked leaf lettuce. Eight larvae, in two replicate groups of four larvae each, were exposed to one liter of lead nitrate, dissolved in spring water, at concentrations of either 0.1 parts per million, 0.5 ppm, 1.0 ppm or 1.5 ppm. One group, consisting of eight untreated larvae, were used as the initial control animals. However, the entire group died within eight days of unknown causes. Consequently, the 0.1 ppm lead treated larvae were substituted as the control group since this concentration is permitted in drinking water. The larvae were observed from early limb bud stage, stage X, through the protrusion of the forelimbs, stage XX (Taylor and Kollros, 1946). Observations were made over a period of 106 days.

In a second series of experiments, two organisms of stage XI (Taylor and Kollros, 1946) were exposed to each experimental concentration of lead nitrate for one week. Stage XI was chosen because it is the beginning stage of prometamorphosis. The larvae were fixed in 50% Bouin's fluid (Carleton and Drury, 1957) for 18 hours at 5°C ± 1°C (Berson, 1972). The head portions were subjected to standard histological preparation and stained with Periodic acid-Schiff and counterstained with Mayer's hematoxylin and eosin (Humason, 1972). Serial sections of the thyroid were studied by light microscopy.

The time required for metamorphosis was expressed as days ± the standard deviation. The significance of the difference between the time required for metamorphosis at different lead concentrations was determined by the student's T test (Simpson et al., 1960).

RESULTS

Lead, at the experimental concentrations, did not alter the sequence of metamorphic stages in *Rana utricularia*. All larvae exposed to experimental concentrations of lead exhibited normal gross external and internal morphology.

The time required for the completion of each successive metamorphic stage was altered, however, by different concentrations of lead (Figure 1). The 0.1 ppm lead treated larvae required 53.0 days to complete stage XX. The 0.5 ppm lead treated larvae required 55.7 days to complete development of stage XX. The 1.0 ppm lead treated larvae required 74.8 days to complete stage XX. The time required by 1.5 ppm lead treated larvae was intermediate (65.5 days) between the time required by 1.0 ppm lead treated larvae and 0.5 ppm lead treated larvae. The extent of delay was shown to be concentration dependent. The differences in time required to complete metamorphosis between the 1.0 ppm lead treated larvae and 0.5 ppm lead

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treated larvae was shown to be significant (P = 0.05 – 0.1) by the student's T test. The differences in time required to complete metamorphosis between 1.0 ppm and 1.5 ppm lead treated larvae and 0.5 ppm and 1.5 ppm lead treated larvae were statistically insignificant because of the insignificance of the difference of the means.

Examination of the serial sections of the thyroid gland revealed histological alterations attributed to lead. The extent of the alterations was also concentration dependent. In the control larvae, the shape of the thyroid follicles is spherical, and colloidal material completely fills the lumen (Figure 2). The epithelium is cuboidal and contains spherical nuclei. No black particles are seen in the epithelial cells of the control. The most extreme alterations of histological features occur in the thyroid of the 1.0 ppm lead treated larvae. Although the epithelium is normal, the follicles are poorly differentiated (Figure 3), and are completely devoid of colloidal material. The size of the thyroid also is reduced, and black particles appear in the gland. In the 0.5 ppm and 1.5 ppm lead treated larvae, the size of the thyroid is reduced but the shape of the follicle and the epithelium are the same as in that of the control. The colloidal material appears to have experienced some shrinkage and contains peripheral vacuoles (Figure 4).

DISCUSSION

In this experiment, *Rana utricularia* larvae of stage X were exposed to lead concentrations of 0.1 ppm, 0.5 ppm, 1.0 ppm, and 1.5 ppm for 106 days. No alteration in the sequence of metamorphosis or in gross external and internal morphology were observed. This result is in contrast to the finding that lead at a concentration of 1.0 ppm causes major defects in chick embryos at hatching Birge and Roberts, 1976). The defects in the chick included brain deficiencies, absence of eyes, skeletal anomalies, and severe motor impairment. Apparently these two species differ greatly in their susceptibility to lead.

Lead prolongs the time of metamorphosis by delaying the completion of each successive metamorphic stage (Figure 1). The extent of delay varies with different lead concentrations. The 1.0 ppm lead treated larvae required the most time to complete each metamorphic stage followed by 1.5 ppm, 0.5 ppm, and 0.1 ppm lead treated larvae. The amount of lead entering the system of the larvae has not been determined in this experiment, although evidence of the presence of lead in tissues is seen. Catizone and Gray (1941) working with chick embryos found no correlation between the strength of solution injected and the number and kind of abnormalities resulting in the central nervous system. In contrast to this, the teratogenesis in chick embryos is claimed by Birge and Roberts (1976) to be concentration dependent and to correlate inversely with survival. Therefore, it may be postulated that a mechanism, as yet undefined, exists, which could control the amount of lead entering the larvae. Since the transport of lead across biological membranes is largely lead concentration independent (Kostial and Momcilovic, 1974). The presence of such a mechanism could possibly aid in the explanation of the resistance to lead by the 1.5 ppm lead treated larvae.

The prolonging effect of lead was more prominent during the time of hindleg growth and development. The developmental rate of the larvae exposed to low concentration of lead, 0.5 ppm, shows that the rate of development is depressed until stage XIV, then the larvae produced through successive metamorphic stages at a faster rate. The developmental rate curves of the larvae exposed to high concentrations of lead, 1.0 ppm and 1.5 ppm, show that the rates were depressed much longer until stage XVIII. These results indicate that lead in high concentrations acts to delay metamorphosis by prolonging the time required for completion of the metamorphic stages, especially during development prior to stage XVIII.

Because of the involvement of the thyroid hormone, thyroxine, in metamorphosis, examination of thyroid histology was made to assess the effects of lead on the tissue. Considerable alteration was noted, many features of which resemble those seen in hyperthyroidism (Bloom and Fawcett, 1964). An expanded investigation of the effect of lead at successive stages of thyroid development is indicated.

Since the characteristics observed in the experimental thyroid, such as black particles, retarded development and vacuolation were not observed in the control, it can be said that these features are

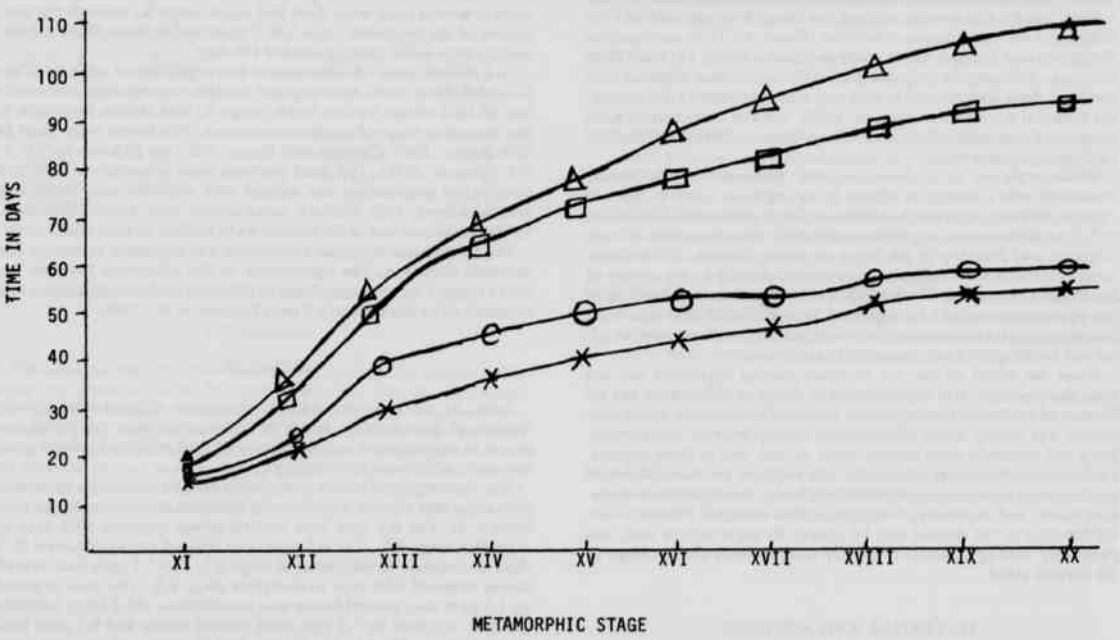

Figure 1. Developmental rate curves of *Rana utricularia* larvae. x-0.1 ppm lead treated larvae; 0-0.5 ppm lead treated larvae; Δ-1.0 ppm lead treated larvae and -1.5 ppm lead treated larvae.

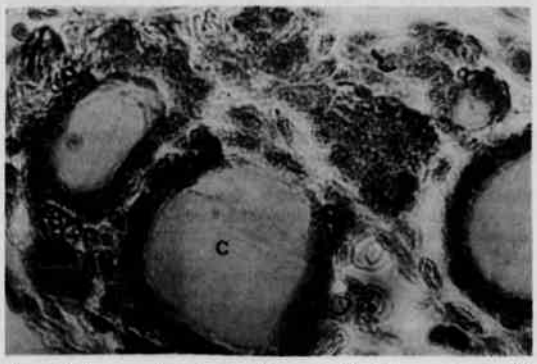

Figure 2. *Rana utricularia* thyroid, control-lead free; stained with alcoholic Periodic acid-Schiff, counterstained with Mayer's hematoxylin and eosin; magnification 375X, C-colloidal material; E-epithelium of thyroid follicle.

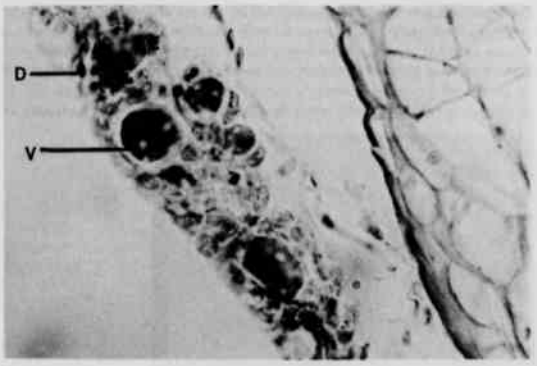

Figure 3. *Rana utricularia* thyroid, exposed to 0.5 ppm lead; stained with alcoholic Periodic acid-Schiff, counterstained with Mayer's hematoxylin and eosin; magnification 375X, C-colloidal material; D-dark particle; E-epithelium of thyroid follicle; V-vacuole.

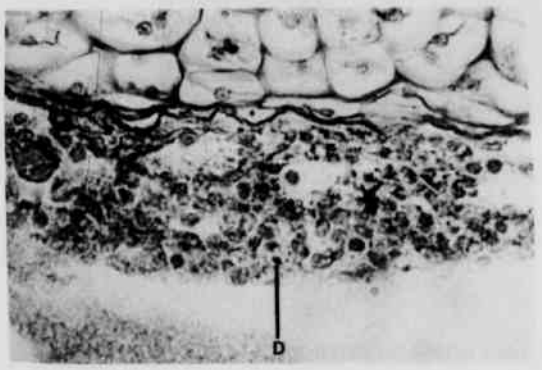

Figure 4. *Rana utricularia* thyroid, exposed to 1.0 ppm lead; stained with alcoholic Periodic acid-Schiff, counterstained with Mayer's hematoxylin and eosin; magnificantion 375X, D-dark particle; E-epithelium of thyroid follicle.

attributed to the action of lead. Lead had been shown to accumulate in fins, gills and liver of the fish (Merlini and Pozzi, 1977) and in kidney, bone and liver of *Xenopus* (Ireland, 1977). It is suggested that the dark particles seen in the thyroid may be lead in some form. The lead which appears to be deposited in the thyroid may impair the function of the thyroid which, in turn, delays metamorphic events. This suggestion is feasible since it has been shown that lead impairs thyroid function both *in vitro* and *in vivo* in rats and in man (National Academy of Science, 1972). *In vitro*, lead impairs the uptake of iodine^{131} by thyroid slices. Also, in lead poisoned rats, the poisoned rats, the uptake of iodine and the conversion of iodine to protein bound iodine are retarded. Therefore, one possible action of lead may be the impairment of iodine uptake. This possibility is further supported by the occurrence of vacuoles in the colloid of the thyroid of both 0.5 ppm and 1.5 ppm lead treated larvae. Similarly, vacuoles occur in the colloid of the thyroid of quails when the birds have been injected with prolactin (Wade et al., 1975). Prolactin has been found to lower significantly thyroidal iodine^{131} uptake in tadpoles (Gona, 1958). This hypothesis does not rule out, however, the possibility that lead also affects the hypothalamus and/or the pituitary which, in turn, may influence the thyroid and the event of metamorphosis.

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