The Activity of △5-3β Hydroxysteroid Dehydrogenase Enzyme in the Interrenal Tissue of Rainbow Trout (*Salmo gairdneri* Richardson) Exposed to Sublethal Concentrations of Zinc

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ABSTRACT

The effects of exposure of rainbow trout (Salmo gairdneri Richardson) to three sublethal concentrations of zinc (0.248, 0.528 and 1.14 ppm) on Δ5-3β-hydroxysteroid dehydrogenase (Δ5-3βHSDH) enzyme activity in the head kidney tissue was investigated. The activity of this enzyme was localized by the reduction of a tetrazolium salt to formazan at the site of activity. Using subjective methods to indicate the degree of activity and indirectly assess the degree of corticosteroid production, the zinc-exposed rainbow trout showed a greater degree of Δ5-3βHSDH activity compared to the control fish. This increase in enzyme activity has been associated with the stimulation of the pituitary-interrenal axis by the noxious stress of zinc.

INTRODUCTION

A number of investigators have used the term "stress" to describe a fish's response to environmental pollutants (CRANDALL and GOODNIGHT, 1962, 1963; HILL and FROMM, 1968; SILBERGELD, 1974; SNIESZKO, 1974; DONALDSON and DYE, 1975; WATSON, 1975). The physiological basis of this "stress" response is considered to be endocrinological, involving primarily the pituitary-interrenal axis. Under conditions of stress the pituitary is stimulated to produce more ACTH resulting in elevated levels of corticosteroids secreted by the interrenal tissue (HILL and FROMM, 1968; SANGALANG and FREEMAN, 1974; DONALDSON and DYE, 1975; WATSON, 1975). To date, histological observations of interrenal tissue and corticosteroid hormone measurement have been used mainly as indicators of interrenal activity (HANE et al., 1966; HILL and FROMM, 1968; BUTLER, 1973; SUBHEDAR and RAO, 1974; DONALDSON and DYE, 1975).

This study has employed a third method of measuring or qualitatively indicating the degree of interrenal activity; the histochemical demonstration of Δ5-3βhydroxysteroid dehydrogenase (Δ5-3βHSDH) enzyme employing the

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procedures outlined by WATTENBERG (1958).

In enzymatic reactions where nicotinamide adenine dinucleotide (NAD) serves as a coenzyme, as in the case of dehydrogenation of Δ5-3β hydroxysteroids via Δ5-3βHSDH, they can be coupled via NAD diaphorase with tetrazolium salt reduction to formazan (WATTENBERG, 1958; WIEBE, 1969; CHESTNUT, 1970). The formazan is therefore deposited at the site of enzyme activity. CHESTNUT (1970) and WATSON (1975) have commented on the value of demonstrating this enzyme in tissue incubations to assess (qualitatively) an organ's steroid synthesizing capabilities. This study was conducted to investigate the effects of sublethal concentrations of zinc on the activity of Δ5-3βHSDH enzyme in rainbow trout interrenal tissue after a long-term exposure.

MATERIALS AND METHODS

The rainbow trout used in this experiment were obtained from the Rainbow Ranch, R.R. 1 Moffat, Ontario, in April 1974 at age 18 months and weighed between 100-150 g. The fish were placed in a 500 l circular fibreglass tank which was provided with running well water at 10± 0.1°C, one submersible circulation pump and air. Lighting in the room was supplied by ceiling fluorescent light fixtures which were controlled by an electric time switch, adjusted weekly to stimulate a natural light period. Fish were fed daily ad libitum on a commercial pellet diet (Martin Feed Mill Co., Elmira, Ontario). The fish were held under these conditions until four weeks prior to the onset of the experiment at which time 8 fish were randomly placed in each of four experimental tanks, anaesthetized in 50 ppm MS222 (Kent Laboratories) and weighed. The experimental tanks used were 50 l enamelled circular tanks so constructed as to allow automatic collection and removal of fecal debris (WATSON, 1975). Analysis of variance (ANOVA) was performed on the weight observations to ensure homogeneity among the four tanks.

During these four weeks, which served as an acclimation period to the tanks, and the experimental period, fish were fed once daily with a volume of food based on 1% of the total weight of fish in each tank. Dissolved oxygen was checked during the acclimation period by the unmodified Winkler method (TARAS et al., 1971) and was never below 7.4 ppm.

At the end of the four-week acclimation period, fish in each tank were again anaesthetized in 50 ppm MS222, weighed and ANOVA performed to ensure that no discrepancies in weight existed among the tanks when the experiment began. The fish in three of the four

tanks were used for exposure to zinc while those in the fourth served as controls. Three diluting apparatuses (WATSON, 1975) delivered 2 l/min of zinc solution to each of these tanks. There was approximately 50 l of aerated water in each tank, with the flow rate described above, 99% replacement was achieved in 2.0 hours. Stock solutions of zinc were prepared by adding zinc sulphate ($ZnSO_4 . 7H_2O$) to distilled water. Fish were exposed in this manner for 50 days, (from June 23 to August 12, 1974). At the end of the exposure period fish were stunned by a blow to the head, weighed and the anterior portion of the head kidney was excised and processed for localization of Δ5-3β-HSDH. ANOVA was performed on the weight observations. The experiment was conducted under a constant 12h light - 12h dark light regime.

Measurements of hardness (EDTA tritimetric method) and dissolved oxygen content (unmodified Winkler method) were carried out according to the methods outlined by TARAS et. al., (1974). Temperature and pH were also recorded regularly.

The concentration of zinc (ppm) was measured every 10 days in each tank. Water samples were collected in 250 ml polyethylene bottles and acidified to pH 5.0 with 1.ON HCl. Zinc concentrations were determined with a Zeiss spectrophotometer (Model PMQ II) equipped with a flame attachment (Model FA II) for atomic flame absorption at a wavelength of 213.85 nm.

The head kidney tissue was immediately frozen in isopentane precooled in liquid nitrogen. Cryostat sections were collected on glass over slips and processed according to the procedures outlined by BAILLIE, FERGUSON and HART (1966) for localization of Δ5-3βHSDH activity. Pregnenolone (Δ5 pregnene-3βol-20-one, Sigma) was used as the substrate since it is a precursor for most steroids (WIEBE, 1969; CHESTNUT, 1970; WATSON, 1975). Table 1 shows the contents and concentration of materials used in the incubation medium. Nicotinamide was used to protect the NAD from enzymatic destruction. Sections were incubated at 37°C for 1 - 2h.

Control sections were incubated: (a) without any substrate precursor (this incubation served as a negative control to eliminate a possibility of a nonspecific reaction in the incubation medium), and (b) with reduced adenine dinucleotide (NADH) to test for diaphorase activity. In addition, sections of rat adrenals were tested concurrently with each head kidney incubation (the rat adrenals in this case served as a positive control for indicating the presence of Δ5-3βHSDH).

TABLE 1

Concentration and contents of materials used in the incubation medium for localization of Δ5-3βHSDH activity in rainbow trout interrenal tissue.

Material	Concentration	Quantity Total (ml)
pregnenolone	0.5 mg/m dissolved in DMF (Sigma)	0.5
buffer medium	0.1M Gomori's buffer pH 7.2-7.4	4.0
NAD or NADH (Sigma)	2 mg/ml in 0.1M Gomori's buffer	1.0
nicotinamide (Sigma)	0.16% in distilled H_2O	1.0
nitro blue tetrazolium (Sigma)	0.5 ml in distilled H_2O	1.0

After incubation of the sections was completed they were mounted on glass slides with glycerine jelly, examined under a microscope, and photographed. In order to semi-quantify the degree of formazan deposition, WATTENBERG'S (1958) scoring scheme was used which assigns a number (0-5) to the intensity of colour. Following is the scoring scheme: negative, 0; pink, 1; red or light purple, 2; purple, 3; dark purple, 4; maximum reaction, 5.

A number of mortalities occurred: control (1); 1.14 ppm zinc-exposed group (3); 0.528 ppm zinc-exposed group (1); and the 0.248 ppm zinc-exposed group (2). The cause of these mortalities was unknown. None of the dead fish were included in any of the statistical calculations.

RESULTS

Table 2 shows the physical-chemical properties of the well water used in this experiment. Table 2 shows the mean weights (±S.E.) of the fish in each tank at day 0 and after 50 days exposure to zinc as well as the observed zinc concentrations (±95% confidence limits). Results of ANOVA on the weight data are also given in Table 3. No significant differences ($P>0.05$) in weight was observed between the control and any of the zinc-exposed fish.

Figures 1-6 are photographs illustrating the results of the head kidney tissue incubations for Δ5-3βHSDH activity in the interrenal cells. One representative photograph from each of the four treatments is presented. The highest mean scores for the degree of formazan deposition (or Δ5-3βHSDH activity) occurred in the three zinc-exposed groups of rainbow trout; 0.248 ppm zinc (2.41), 0.528 ppm zinc (2.21), 1.14 ppm zinc (2.0) with the control's mean score of 1.29 being the lowest.

TABLE 2. Physical-chemical properties of the well water used in this experiment.

Tank	Oxygen Concentration Range (mg/l)	N	Temperature °C ±S.E.	N	pH* ±S.E.	N	Hardness (EDTA) Range (mg/l)	*N
1 (control)	5.8-9.9	5	10.0±0.1	5				
2 zinc exposed	6.9-8.0	5	10.0±0.1	5	7.3±0	5	364.0-400.0	5
3 zinc exposed	6.6-8.2	5	10.0±0.1	5				
4 zinc exposed	5.6-7.4	5	10.0±0.1	5				

* Determinations made from one tank

TABLE 3. Mean weights (±S.E.) of fish at day 0 and after 50 days exposure to zinc, and the observed zinc concentrations (±95% confidence limits).

Tank	Mean Weight ±S.E. (g) Day 0	Mean Weight ±S.E. (g) After 50 days	Mean Obscured Zinc Concentration (mg/l)	95% Confidence Limits (mg/l)	N
1 control	*130.9± 9.0	*168.7±12.6	0.1	————	5
2	*142.0±10.7	*170.6±21.4	0.248	± 0.013	5
3	*150.1± 8.3	*176.8±18.2	0.528	± 0.02	5
4	*133.6±10.5	*172.3±15.9	1.14	± 0.21	5

* Not significant at P>0.05

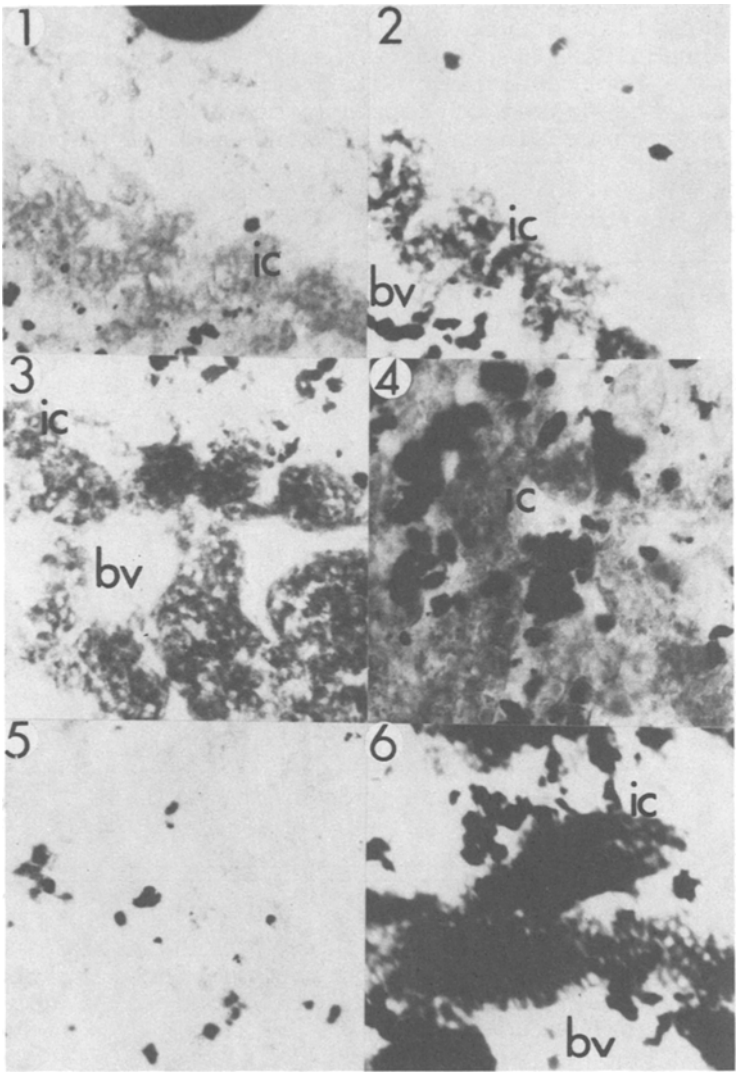

Fig.1 Control rainbow trout head kidney incubation for Δ5-3βHSDH activity showing a lesser degree of activity than the three zinc-exposed groups (200x).

Fig.2 0.248 ppm zinc-exposed rainbow trout head kidney incubation for Δ5-3βHSDH activity showing a greater degree of activity than the control (220x).

Fig.3 0.528 ppm zinc-exposed rainbow trout head

kidney incubation for Δ5-3βHSDH activity showing a greater degree of activity than the control (200x).

Fig.4 1.14 ppm zinc-exposed rainbow trout head kidney incubation for Δ5-3βHSDH activity showing a greater degree of activity than the control (200x).

Fig.5 Head kidney incubation without substrate pregnenolone, this served as a negative control, no Δ5-3β-HSDH activity is present (200x).

Fig.6 Head kidney incubation with NADH showing diaphorase activity in a group of interrenal eels (200x). ic - interrenal cells bv - blood vessel

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DISCUSSION

ANOVA of the weight observations of rainbow trout after a 50-day sublethal exposure to 0.248, 0.528 and 1.14 ppm zinc indicate that growth was not affected. Although a number of authors have reported growth inhibition of fish in response to sublethal concentrations of zinc their experiments were conducted over a considerably longer time and were specifically designed as growth experiments (BRUNGS, 1969; BENGTSSON, 1974; WATSON, 1975). The lack of apparent growth differences between the control and zinc-exposed rainbow trout in this experiment are likely a function of the experimental design and do not imply that similar findings would occur in longer-term experiments.

Results of the interrenal histochemical localization of Δ5-3βHSDH activity shows that this enzyme's activity is increased in vivo in response to sublethal levels of zinc. Since this enzyme is important in the synthesis of most biologically active steroids (WIEBE, 1969) from Δ5-3βhydroxy steroids, its demonstration can be an invaluable aid in subjectively assessing the state of the steroid synthesizing activities of interrenal cells.

Cortisol and corticosterone are the major corticosteroids synthesized in the interrenal tissue of rainbow trout; cortisol being the most important physiologically (IDLER, 1972; BUTLER, 1973). Although androgens and estrogens are also synthesized in rainbow trout head kidney tissue, their contribution to the formazan depositied here would be minimal since they are synthesized in very low quantities in this tissue (IDLER, 1972). Therefore, most of the Δ5-3βHSDH enzyme activity observed in these sections can be attributed almost entirely to the ultimate synthesis of cortisol and corticosterone.

Elevated levels of plasma corticosteroids found in fish exposed to environmental toxicants and during certain stressful periods of their life cycle have been described as the animals' "stress response" attributed to the stimulation of the pituitary-interrenal axis (HANE et al., 1966; HILL and FROMM, 1968; BUTLER, 1973; SANG-ALANG and FREEMAN, 1974; DONALDSON and DYE, 1975). Therefore, if one were to assume a similar response in the rainbow trout of this experiment exposed to zinc resulting in elevated levels of corticosteroids, then one would also expect an increase in the amounts of the enzymes associated with their synthesis. Results of this experiment seem to indicate that this is the case by the increase in concentration of Δ5-3βHSDH enzyme permitting greater quantities of steroid substrate material to be synthesized to cortisol and corticosterone when rainbow trout are exposed to sublethal levels of zinc.

The hypothesis that the pituitary-interrenal axis in rainbow trout is stimulated by the noxious stress of zinc seems to coincide with the evidence presented here.

Although the methods employed for assessing Δ5-3β-HSDH enzyme activity in this experiment are subjective they are, nevertheless, quite useful to indicate the relative degree of activity of Δ5-3βHSDH enzyme. They also provide an indirect assessment of the degree of corticosteroid production in rainbow trout interrenal tissue.

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