

***IN VITRO* METABOLISM OF PREGNENOLONE BY RAINBOW  
TROUT EMBRYOS AND THE IDENTIFICATION OF ITS  
METABOLITE**

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**Introduction**

In oviparous species, it is generally accepted that steroids are passively incorporated into oocytes during follicular growth. Since yolk is the sole source of nutrients for oviparous species prior to exogenous feeding, there is the possibility that salmonid embryos are exposed to these maternal yolk steroids,

which, if not metabolized, pose a potential hazard to the developing embryo. Some studies suggested that steroid hormones play specific functions during early development (Yeoh et al., 1996; Khan et al., 1997). However, studies related to gonadal steroid hormone metabolism in fish embryos have focused on the biologically potent androgens and estrogens (Yeoh et al., 1996; Khan et al., 1997). Much less is known about the metabolism and possible role of pregnenolone (P<sub>5</sub>). Thus, the purpose of this study was to examine if P<sub>5</sub> metabolism occurs during embryogenesis, and if so, which metabolic pathways might be present at different developmental stages. The study is part of an ongoing investigation into the processes of steroid metabolism in fish embryos, and of the nature of intermediate steroid metabolites, and an evaluation of the toxicological implications if these metabolic pathways are influenced by xenobiotics.

### Methods

Whole rainbow trout embryos (minus the yolk) were incubated *in vitro* in 24 well-tissue culture plates containing 2 ml of medium M199 at the presence of radiolabelled P<sub>5</sub> ([<sup>3</sup>H] P<sub>5</sub>) at 8-10°C for 18 h. At termination, the media was collected and extracted using a solid-phase extraction, Sep-Pak C<sub>18</sub> cartridge. Free and conjugated fractions, following acid solvolysis and glucuronidase treatments, were subsequently separated using a reverse-phase High Performance Liquid Chromatography (HPLC), as previously described (Khan et al., 1997).

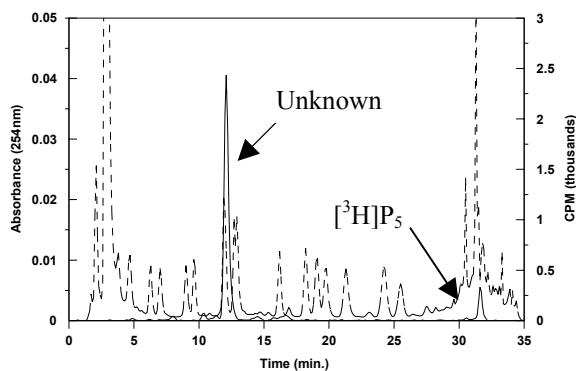
An identification of unknown compound(s) was carried out using a Gas Chromatography and Mass Spectrometry (GC-MS). The unknown metabolite from *in vitro* incubation, as previously explained, of rainbow trout embryonic tissue in the presence of non-radiolabelled P<sub>5</sub> was collected, derivatized, and subsequently identified using GC-MS (Condeca and Canario, 2001).

### Results and Discussion

P<sub>5</sub> was converted mainly to an unknown metabolite in the free fraction (Figure 1) with much less conjugated metabolites.

Figure 1. A representative HPLC profile following the *in vitro* incubation of yolk-absorbed embryos (63 days post fertilization) in the presence of [<sup>3</sup>H]P<sub>5</sub> at 8-10°C for 18 h. The solid line represents radioactivity (count per minute), and the dotted line represents authentic standards (optical density).

The mass spectrum of the unknown compound eluted at 11.5 min was very similar to the authentic standard,  $7\alpha$ -hydroxypregnenolone ( $7\alpha$ -OHP<sub>5</sub>). The similarity of the indices designated fit, reverse fit and purity were 977, 831 and



816 respectively, which was higher than the identification criteria of 900, 600 and 600 respectively (Condeca and Canario, 2001).

Although the conjugated steroids represented only a minute fraction in this experiment, there was a mark difference in that of the conjugated patterns. None of  $[^3\text{H}]P_5$  was found in the glucuronide fraction whereas  $[^3\text{H}]P_5$  and  $7\alpha$ -OH $[^3\text{H}]P_5$  were present in the sulfate form. This suggests that sulfation may be preferred for  $[^3\text{H}]P_5$ . However, the possibility exists that the amount of  $[^3\text{H}]P_5$  may be too low to provide sufficient substrate for the glucuronidation in competition with sulfoconjugation. The additional glucuronide of  $7\alpha$ -OH $[^3\text{H}]P_5$  may imply that structural changes due to  $7\alpha$ -hydroxylation favor glucuronide conjugation. In addition to our result,  $7\alpha$ -hydroxylated steroids in fish have been reported earlier (Kime et al., 1991, Ponthier et al., 1998), but there is no strong evidence regarding its biological role. Taken together, the  $7\alpha$ -hydroxylation of  $[^3\text{H}]P_5$  and the additional glucuronide of  $7\alpha$ -OH $[^3\text{H}]P_5$  may imply the excretion pathway of  $[^3\text{H}]P_5$  by rainbow trout embryos. Whether or not the  $7\alpha$ -OHP<sub>5</sub> has a physiological role in fish is not clear.

## Conclusion

The study shows the production of  $7\alpha$ -OHP<sub>5</sub>, a novel steroid, by fish embryos. Based on the conjugation pattern in our study, we hypothesize that  $7\alpha$ -OHP<sub>5</sub> is a possible route of excretion of P<sub>5</sub>. However, based on evidence of protective roles mammalian neurosteroids, a novel function of  $7\alpha$ -OHP<sub>5</sub> cannot be ruled out.

## Acknowledgements

This study was supported by NSERC and OMAFRA granted to John Leatherland and a postgraduate fellowship from the Royal Thai Government to Rakpong Petkam. We thank the staff at Alma Aquaculture Research Station, Alma, University of Guelph, Ontario, Canada for their invaluable assistance in this research.

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