Metabolism of Oestradiol-17β by Male and Female Larvae of *Xenopus laevis*

Earlier investigations in our laboratories have shown that male and female larvae of *Xenopus laevis*, at a very early age (9–17 days old; stages 25b–25d)[1], are capable of oxidizing testosterone and oestradiol-17β to an appreciable extent[2]. These findings indicated that larvae of *X. laevis*, during the phases of their gonadal development, already possess the enzymes necessary for the metabolism of steroid hormones (17β-hydroxysteroid oxidoreductases; hydroxylases). The male larvae preferentially oxidize and thereby inactivate the female hormone, whereas the female larvae primarily metabolize the male hormone[2]. This communication deals with the identification of 3 metabolites of oestradiol-17β by both male (*ZZ*) and female (*ZW*) larvae of *X. laevis*.

The production of pure cultures of either the *ZZ* male or the *ZW* female genetic constitution has been described in detail[3]. In brief, the method originally consists in converting genetic males into females and genetic females into males. The unisexual *ZZ* or *ZW* cultures are obtained by breeding such converts. They are not themselves received by sex reversal.

All experiments were carried out with 17-day-old larvae; they were starved for 24 h before incubation. 50 male and 50 female larvae were collected and separately incubated with 5 μC of [4-^{14}C]oestradiol-17β (sp. act. 53.4 mC/m*M*) in 500-ml flasks, containing 250 ml of water. The radioactive steroid was added in ethanolic solution to the incubation medium; the final concentration of ethanol did not exceed 0.5%. To prevent bacterial contamination, 10,000 units of penicillin were added $^1/_2$ h before the addition of [4-^{14}C]oestradiol-17β. During the incubation period, the aqueous medium was continuously aerated. After 24 h of incubation, the contents of the flasks (water and larvae) were extracted 3 times with an equal volume of distilled ether. The combined ether extracts were evaporated to dryness under vacuum, and the residue was dissolved in methanol-benzene (1:1, v/v). Aliquots were subjected to paper chromatography in the system methanol-water-petroleum ether-benzene (33:40:17:10, v/v)[4]. 5 μg of oestriol, oestradiol-17β and oestrone were run as parallel standards. The radioactivity on the paper was measured in a Packard Radiochromatogram scanner, model 7200, and the oestrogen standards were located with Folin-Ciocalteus reagent[5].

After paper chromatography in the above system, 2 peaks of radioactivity (P-1 and P-2) were observed. P-1 was more polar than oestradiol-17β and oestriol, whereas P-2 corresponded in polarity with oestrone; in none of the experiments, any radioactivity was found in the position of oestradiol-17β. The 2 peaks were eluted separately with methanol and rechromatographed on propylene glycol-impregnated paper with toluene. P-2 was chromatographed for 7 h and again showed the same mobility as authentic oestrone. P-1 was run for 20 h and had a mobility similar to, but not identical with oestriol. Since it is well known that in many paper chromatographic systems the position of oestriol contains also other hydroxylated oestrogens, the oestriol zone was eluted and rechromatographed for 15 h on formamide-impregnated paper with chloroform, using the following steroids as standards: oestriol, 16-*epi*-oestriol, 17-*epi*-oestriol, 16-oxo-oestradiol-17β and 16α-hydroxyoestrone. After scanning the chromatogram, 3 distinct peaks (P-1a, P-1b and P-1c) were detected; the most polar peak (P-1a) was not further investigated. P-1b corresponded in mobility with oestriol and P-1c with 17-*epi*-oestriol. For further identification, P-1b was eluted and chromatographed on formamide-impregnated paper in the system chloroform-ethyl acetate (5:1, v/v)[6]; a single peak of radioactivity was obtained, corresponding exactly with authentic oestriol. P-1c was subjected to chromatography on formamide-impregnated paper in the system monochlorobenzene-ethyl acetate (3:1, v/v)[6]; this chromatography yielded 2 radioactive peaks whose positions were identical with authentic 6α-hydroxyoestrone and 15α-hydroxyoestrone, respectively.

The quantitative results are summarized in the Table. As already mentioned, no [4-^{14}C]oestradiol-17β was recovered in both experiments, thus indicating a vigorous metabolism of oestradiol-17β by larvae of *X. laevis*. Significant differences between male and female larvae were observed in the formation of oestrone from oestradiol-17β. Furthermore, oestrone is hydroxylated by male larvae to a greater extent than by the female larvae. The over-all formation of oestrone and its hydroxylated derivatives by the male larvae is 1.5 times greater than that for the female larvae.

The present findings show that male and female larvae of *X. laevis*, long before the onset of metamorphosis, are capable of converting oestradiol-17β not only to oestrone, but also to at least 3 additional metabolites, 1 of which (15α-hydroxyoestrone) has been found so far only in mammals[7,8].

Zusammenfassung. Nach 24 h Inkubation von [4-^{14}C]-Östradiol-17β mit 17 Tage alten männlichen und weiblichen Larven des Krallenfrosches (*Xenopus laevis*) unter In-vivo-Bedingungen wurden folgende radioaktive Metaboliten papierchromatographisch nachgewiesen: Östron, 6α-Hydroxyöstron, 15α-Hydroxyöstron und Östriol.

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Amounts of metabolites formed during incubation of [4-^{14}C]oestradiol-17β with 17-day-old larvae of *Xenopus laevis*

Sex	% of radioactivity identified as			
	Oestrone	Oestriol	6α-Hydroxy-oestrone	15α-Hydroxy-oestrone
ZZ♂	1.3	7.9	7.6	12.8
ZW♀	2.4	7.4	6.4	5.0

The figures in the Table are calculated on the basis of radioactivity, recovered in the ether extract at the end of 24 h of incubation.

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