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Estrogens and estrogenic effects in *Tilapia aurea* (Cichlidae, Teleostei)

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Summary. Estradiol (E_2) level in plasma of non-breeding female *Tilapia aurea*, measured by RIA, was 0.33 $\pm$ 0.02 ng/ml (mean $\pm$ SEM, n = 6). This level is about 10 p. 100 of the level found in breeding females, about 4 times higher than in ovariectomized females and similar to the level found in non-breeding males.

Twenty-four hours following a single i. p. injection of 0.5 mg E_2, the estradiol level in plasma of ovariectomized *Tilapia* was within the physiological range (0,2-10 ng/ml). This level was maintained for at least 4 days. Comparison of the vitellogenic potency of 3 estrogens in ovariectomized *T. aurea* shows that estriol was more potent than estradiol, which, in turn, was more potent than estrone. Since E_2 increased in the plasma of estrone-injected fish, it is assumed that estrone was converted to estradiol in the recipient fish.

Introduction.

Raising water temperature above 22° induces ovarian growth and spawning in *Tilapia aurea* (Fishelson, 1966). By employing the isotopic derivative technique it was shown that in female *T. aurea* exposed to 30° the plasma level of testosterone, 11-ketotestosterone, 11β-hydroxytestosterone and deoxycorticosterone (DOC) is considerably higher than in females kept at 18° (Katz and Eckstein, 1974). The increase of DOC level was explained by its possible role in the maturation of oocytes and ovulation, in line with the findings of Goswami and Sundararaj (1971) in *Heteropneustes fossilis*. However, the increased level of the three androgens remained unexplained. Also estrone (E_1) and estradiol-17β (E_2) were isolated and identified in ovarian extracts of this fish, but they could not be detected in the peripheral plasma, using the isotopic derivative method (Katz *et al.*, 1971 ; Katz and Eckstein, 1974).

Studies on the functional significance of ovarian steroids in bony fishes have been hampered for a long time by the peculiar morphological organization of the ovary and oviduct. « Spaying a female teleost means in most cases that not only the source of the hormones is removed, but also an important part of the potential target organ. » (Reinboth, 1972.) However, E_2 is known to induce the synthesis and secretion of vitelloproteins by the liver in teleosts, as in other non-mammalian vertebrates (Chester-Jones *et al.*, 1972 ; Campbell and Idler, 1976 ; Emmersen and Emmersen, 1976).

In a recent report from our laboratory (Yaron *et al.*, 1977), we have shown that the plasma of *Tilapia aurea* contains immuno-reactive estradiol-17β (3.1 ± 0.7 ng/ml ; mean ± SEM ; n = 26). The specificity of the antibody employed in our radioimmunoassay (RIA) was examined by cross-reaction with all the steroids previously reported in this species. The highest cross-reaction with E_2 was of E_1 (1.4 p. 100). However, the similarity of E_2 determinations in whole versus chromatographically-separated plasma extract indicated that neither E_1 nor estriol (E_3), if present in the plasma of this fish, could contribute to the E_2 determinations. A positive correlation was found between plasma E_2 level and ovarian weight. The plasma E_2 level dropped to 0.08 ± 0.04 ng/ml nine days after ovariectomy. Concentrations of plasma calcium and proteins, indirect parameters of plasma vitelloprotein level, decreased following ovariectomy but could be restored to the initial level by injection of E_2.

This paper presents data on E_2 level in plasma of non-breeding females and males, compares the potency of E_1 and E_3 with that of E_2 on the above-mentioned vitellogenic parameters, and describes the disappearance of injected estradiol from the circulation of ovariectomized *Tilapia*.

Materials and methods.

Fish source, fish maintenance, surgery, blood sampling, protein, calcium and E_2 determinations were as described earlier (Yaron *et al.*, 1977). Plasma E_2 was measured by RIA using the rabbit anti-17β-estradiol-6-BSA-serum. For details on the technique, specificity and validity of the assay, the reader is referred to the latter article. Results are expressed as group means ± SEM.

Results and discussion.

E_2 level in non-breeding T. aurea. Plasma E_2 level was measured in 6 fish of each sex collected after the breeding season (October) and kept for 10 days at 17° and photoperiod of 12 L/12 D. The E_2 and protein levels were similar in both sexes ($p > 0.05$), but calcium level in the plasma of the females was higher than that of the males ($p = 0.044$, table 1).

TABLE 1

Estradiol, calcium and protein in plasma of non-breeding Tilapia aurea [a]

Sex	E_2		Ca		Protein	
	n	ng/ml	n	mg p. 100	n	gr p. 100
females	6	0.330 ± 0.02	6	13.64 ± 0.80	6	4.05 ± 0.28
males	6	0.516 ± 0.086	7	11.88 ± 0.21 [b]	7	4.33 ± 0.24

[a]) Fish were collected after the breeding season (October) and kept at 17 °C for 10 days.
[b]) Significantly different from the female value ($p = 0.044$). Student's t-test.

The E_2 level in non-breeding female *T. aurea* is about one-tenth of the level recorded in females kept at 25° during the breeding season (3.1 $\pm$ 0.75 ng/ml), but 4 times higher than that found in ovariectomized fish (0.08 $\pm$ 0.04 ng/ml). The calcium concentration in the non-breeding females was higher than in males (table 1) and in ovariectomized fish (11.4 $\pm$ 0.2 mg p. 100 ; Yaron *et al.*, 1977). It is assumed, therefore, that E_2 is secreted by the ovary of non-breeding *T. aurea* and that their higher Ca level indicates the presence of vitelloprotein in the plasma even beyond the breeding season.

Disappearance of injected E_2 from circulation. — In many instances a relatively high dose of estrogen was required to elicit a pronounced vitellogenic effect. Since the physiological level of estrogen is rather low, such high doses as used in experimental procedures were justly criticized (Reinboth, 1972). The following experiment was performed in order to clarify the circulatory level of injected estradiol in *T. aurea*. For this study we used ovariectomized *Tilapia* of about 150 g body weight in which E_2 level was lower than 1 ng/ml. Plasma levels of E_2 were measured 5, 24, 120 and 315 h following a single i. p. injection of 0.5 mg E_2 dissolved in sesame oil.

Five hours after the injection, plasma E_2 was extremely high (fig. 1). Between 24 h and 120 h the level of the exogenous steroid was within the physiological range, comparable to the levels encountered in female *Tilapia* with a gonadosomatic index of 1.8 to 4.0 (Yaron *et al.*, 1977). The long intervals between samplings did not permit the calculation of the half-life for this steroid. It is obvious, however, that following an i. p. injection of 0.5 mg to *Tilapia* the « pharmacological » level is sustained less than 24 h, while in the 4 subsequent days the E_2 is maintained at a physiological level.

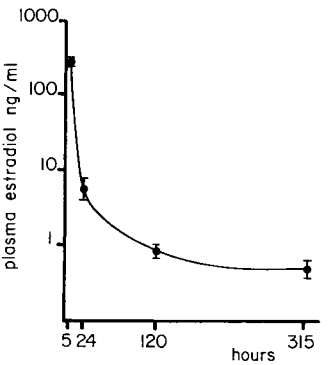

FIG. 1. — *Disappearance of injected estradiol-17β from the circulation of* Tilapia aurea. Estradiol-17β (0.5 mg, 1.2×10^{-5} mole/kg bw) dissolved in sesame oil (0.2 ml) was injected i. p. to 8 ovariectomized fish one month post operation.

Plasma E_2, Ca and protein following the injection of E_1, E_2 or E_3. — This experiment was designed to compare the estrogenic effects in *T. aurea* of three C_{18} steroids known as estrogens in various vertebrates (Chester-Jones *et al.*, 1972). E_1, E_2, E_3 or the vehicle (1.3 ml/kg) were injected i. p. (1.3×10^{-5} mole/kg bw) to female *T. aurea* one month after ovariectomy. Injections were repeated on days 3, 6, 10 and 13. Blood was sampled before the first injection, on day 10 before injection, and on day 18. Plasma calcium and protein were determined as indirect parameters of the vitellogenic response to the injected estrogens (Yaron *et al.*, 1977). The results of day 10 and day 18 were averaged for each fish. For each pair of treatments, the means of these averaged

levels were compared using Student's t-test (unequal variances). The highest vitellogenic response was found in fish treated with E_3. Treatment with E_1 was followed by an increase of the plasma calcium but not of protein. The effect of E_2 on these parameters was significant and similar to that reported earlier in this fish (Yaron *et al.*, 1977) (fig. 2*a*, *b*).

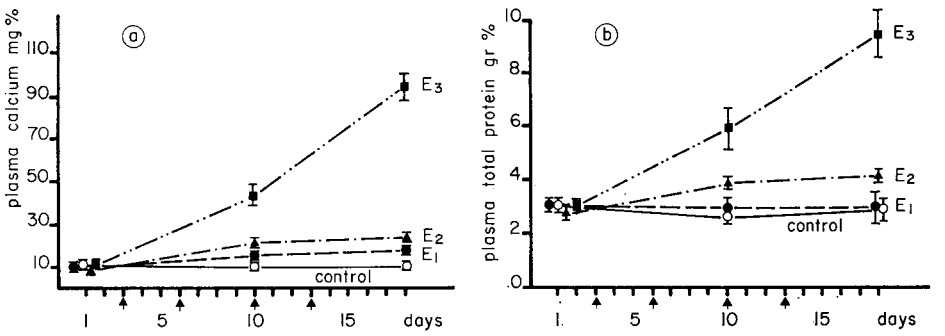

FIG. 2. — *Vitellogenic parameters in* Tilapia aurea *injected with estrone, estradiol or estriol.* The steroids (1.3×10^{-5} mole/kg bw) dissolved in sesame oil (1.3 ml/kg) were injected i.p. to ovariectomized females (*ca.* 150 gr bw) on days marked with arrows. Eight fish in each group. On day 10 blood was sampled before the injection. *a*) Plasma calcium ; *b*) Plasma protein.

In order to ascertain whether the vitellogenic effects of the steroids investigated resulted from the injected substances *per se*, the level of the immuno-reactive E_2 was measured in the plasma of fish from all groups (fig. 3). E_2 level in the plasma of oil-injected, ovariectomized fish remained low throughout the experiment. Similar levels were found in E_3-treated fish. It was not surprising to find higher E_2 levels in plasma of E_2 injected fish, but unexpectedly, the plasma E_2 level of E_1 injected fish increased significantly at day 10 and was similar to the level found in E_2-injected fish. This may indicate a conversion of the injected E_1 to E_2. The inter-conversion between these two steroids is a common phenomenon in vertebrate tissues (Ozon, 1972). If, indeed, the injected E_1 was promptly transformed into E_2 it would be difficult to explain

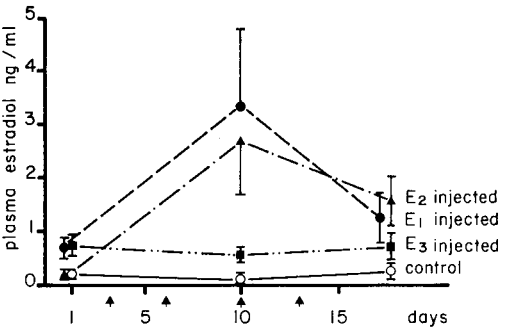

FIG. 3. — *Plasma level of immuno-reactive estradiol in fish injected with* E_1, E_2, E_3 *or oil.* Details as in figure 2.

the low vitellogenic response to the injected steroid. We assume, therefore, that the increase of plasma E_2 in E_1-treated fish resulted from either : (i) a low rate of clearance and a consequent accumulation of E_1 in the plasma (a level of *ca.* 200 ng/ml E_1 may be measured as *ca.* 3 ng/ml E_2 in the RIA used, due to a 1.4 p. 100 cross reaction) ; (ii) a conversion of estrone to an unknown steroid with an affinity to the antiserum similar to that of E_2 but with a low vitellogenic potency ; (iii) the main conversion of E_1 to E_2 occuring close to day 10 after injection, and thus not yet allowing the manifestation of the vitellogenic response ; or (iv) a combination of these possibilities. Nevertheless, the high vitellogenic potency of E_3 may be attributed to this steroid *per se*, since no increase in plasma E_2 was detected in this group. It should be emphasized, however, that estriol has never been reported in *Tilapia aurea*.

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Résumé. Le niveau d'estradiol (E_2) dans le plasma de *Tilapia aurea* femelles en dehors de la période de reproduction, mesuré par dosage radioimmunologique, atteint 0,33 $\pm$ 0,02 ng/ml (moyenne $\pm$ SEM, n = 6). Il représente à peu près 10 p. 100 du niveau trouvé chez les femelles au cours de cette dernière période ; il est environ 4 fois plus élevé que chez les femelles ovariectomisées et égal au niveau trouvé chez les mâles en dehors de la saison de reproduction.

Vingt-quatre heures après une seule injection intrapéritonéale de E_2 (0,5 mg), la concentration plasmatique d'estradiol de *tilapia* ovariectomisées est de l'ordre de celle trouvée chez les femelles normales (0,2-10 ng/ml). Ce niveau se maintient au moins 4 jours. La comparaison de l'effet vitellogénique des trois estrogènes chez les *Tilapia aurea* ovariectomisées montre que l'estriol est plus actif que l'estradiol, et ce dernier plus actif que l'estrone. Puisque E_2 a augmenté dans le plasma de poissons ayant subi une injection d'estrone, ce composé a probablement été converti en estradiol par les poissons injectés.

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