Plasma Androgen Levels in Intact and Castrate Rainbow Trout, *Salmo gairdneri*[1] (36600)

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Androgen assays used for fish blood heretofore required lengthy analytical procedures and large, often pooled quantities of sample. Although techniques used for salmonid fish (1–4) may define some specific steroids, such methods are limited by sampling error, necessity to use large fish, or to pool samples from small specimens. To overcome some of these problems we have modified a competitive protein binding assay proposed for androgens (5) for use in fish. Rainbow trout can synthesize the androgens testosterone, androstenedione, 11β-hydroxytestosterone and 11-ketotestosterone (6). Testosterone, and to a lesser extent other androgens, can competitively displace labeled testosterone from protein in the assay, but estrogens are only weakly competitive and at low concentration in salmonids (7).

To further our knowledge of reproductive endocrinology of teleostean fishes we have assessed the influence of castration on plasma androgen levels in adult, hatchery-reared rainbow trout (*Salmo gairdneri*) and in intact, maturing fish of both sexes.

Materials and Methods. Blood was obtained from male and female fall-spawning, 19-month-old, hatchery rainbow trout in July, 1970, as these fish were nearing sexual maturity. Testicular development in this species occurs earlier than ovarian development. Another group of 27-month-old fish of similar source, kept under a constant light and water temperature regime were either castrated or laparotomized. Sex of the fish was determined at surgery or, in the developing fish, at necropsy.

Castration procedures were similar to Robertson's (8) by midventral incision (ca. 4–5 cm), followed by complete removal of both gonads. Gonads of the sham castrates were examined but not removed. Penicillin G (200,000 units) was placed in the body cavity and the incision was closed with nylon sutures. Furacin (an antibacterial) was applied to the wound. The fish were maintained under light tricaine methanesulfonate anesthesia during the 20 min operation, and water was continuously pumped over the gills. No postoperative mortality occurred.

Fish from the castration experiment were bled 7 days before and at 21 and 42 days after surgery. Blood from all fish was obtained by cardiac puncture with a 21 gauge, 1 in. needle into a heparinized syringe. Plasma was separated by centrifugation and stored at $-15°$ until analyzed.

Plasma (0.1 ml) was extracted with 2 ml chloroform (spectrophotometric grade) with vigorous agitation (vortex mixer). The chloroform layer was removed with a disposable Pasteur pipette and the extraction procedure was repeated with 2 ml of fresh chloroform. The combined chloroform extracts in 15 $\times$ 85 mm disposable culture tubes were evaporated to dryness at 40° under a stream of nitrogen.

Competitive protein binding was carried out to determine the concentration of androgen in the extract. Over 95% of ^{3}H-labeled testosterone added to plasma could be recovered in the extraction procedure, and no attempts to adjust recovery were made. The contents of each tube were shaken with 1 ml

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TABLE I. Mean Androgen Levels in 19-Month-Old Rainbow Trout Nearing Maturity.[a]

	ng/ml
Male	4.6(2), 7.7(1), 8.2(3), 8.9(3), 11.3(2), 11.5(3), 15.9(3), 16.3(2), 31.4(3)
	$\bar{x} = 12.9 \pm 2.6$
Female	3.2(2), 5.1(3), 5.5(3), 8.2(3), 8.6(3), 9.1(3), 9.2(3), 9.4(3), 13.1(3), 17.8(2)
	$\bar{x} = 8.9 \pm 2.8$

[a] Values in parentheses are no. of assays per individual fish; ± refers to standard error of the mean ($\bar{x}$).

of sex hormone-binding globulin (SHBG) saturated with ^{3}H-testosterone [10 ng 1,2-^{3}H-testosterone in 100 ml of 0.3% human late pregnancy (third trimester) plasma in 100 ml deionized water]. Tubes were shaken gently, placed in a water bath at 45° for 5 min and transferred to an ice bath. After 10 min incubation in the cold, 80 mg water-washed, dried (100°) Florisil (60-100 mesh, Sigma Chemical Co.) were added to each tube, shaken for 30 sec and returned to the bath for 3 min. Immediately thereafter 0.5 ml of the supernatant was transferred to a liquid scintillation vial. To the contents of the vial were added 10 ml of scintillation fluid (Aquasol, New England Nuclear) and the radioactivity was counted in a Beckman LS 200 spectrophotometer. The level of plasma androgen was determined by use of a standard curve based on the percentage of counts displaced from SHBG saturated with ^{3}H-testosterone by authentic testosterone at 0, 1, 2, 3 and 4 ng/ml. Student's t and paired t tests were used where appropriate.

Results and Discussion. Differences in androgen levels between maturing male and female trout were not significant (Table I). Although the values for males were much higher on the average, the range between fish in each sex was very wide. Androgen levels in sockeye salmon, *Onchorhynchus nerka*, have been reported to be similar between sexes (1, 3). It is not known to what extent the androgen levels vary in trout at maturity but the ripe males from the same stock in the castration experiment had over twice as much androgen (27.4 ± 5.4) as the less mature males shown in Table I. As in the trout, androgen levels are higher in mature than in immature salmonids of other genera (1, 3, 4).

In the castration experiment the fish were sampled at 21 days after castration because we encountered mortality due to attempted

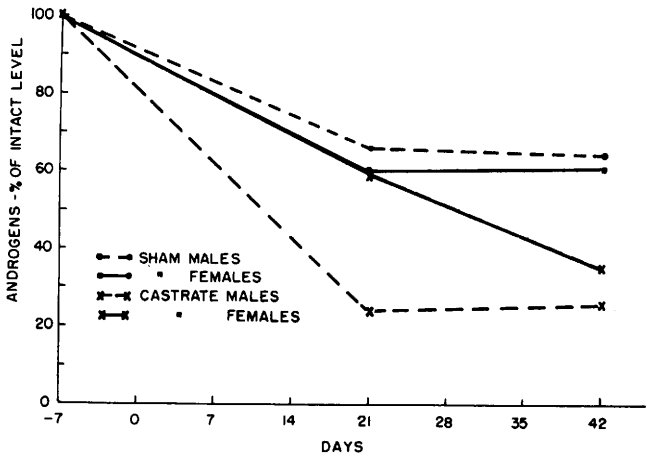

FIG. 1. Androgens in castrate and sham-castrate trout. Surgery was performed on day 0.

bleedings at 7 and 14 days postgonadectomy. At 21 days postcastration androgens decreased ($p < .01$) in males from 27.4 ng/ml before to 6.8 ng/ml after orchiectomy (Fig. 1). No difference was found for ovariectomized fish at this time but at 42 days after gonadectomy, androgen levels decreased from 13.2 ± 1.2 to 4.8 ± 0.4 in the females and remained at about the 21 day level in the males. It appears that the male fish achieved a base level of androgens by 21 ($p < .05$) days while the female fish required 3 more weeks ($p < .05$). In fish of both sexes there is a considerable level of androgens of extragonadal (possibly interrenal) origin.

The clearance rate of gonadal androgens after castration cannot be determined since we analyzed no early postcastration blood samples; however, it is apparent that males clear androgens from blood more readily than females. In the male, this could be due to: (1) a higher demand for androgens by accessory sex structures; (2) a higher rate of androgen excretion; or (3) a lower nongonadal supply of androgens.

Summary. Androgen levels in mature male and female rainbow trout were higher than in maturing fish. Castration reduced plasma androgens in the male more rapidly than in the female.

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