Reprinted from General and Comparative Endocrinology, Volume 21, Number 1, August 1973

GENERAL AND COMPARATIVE ENDOCRINOLOGY 21, 60–68 (1973)

Uptake of ^{3}H-Testosterone and Influence of an Antiandrogen in Tissues of Rainbow Trout (*Salmo gairdneri*)[1]

CARL B. SCHRECK

Colorado Cooperative Fishery Unit, Colorado State University, Fort Collins, Colorado 80521

Received August 7, 1972

^{3}H-Testosterone was administered to yearling rainbow trout (*Salmo gairdneri*) of both sexes to establish accumulation and retention of androgen by various organs and tissues. Radioactivity in plasma, liver, and gonad were determined six times during the 24 hours post treatment. Radionuclide uptake in plasma was rapid, while the retention time in the liver was several times that of the blood, and no sexual dimorphism was found. The testes accumulated significantly more radionuclide than did ovaries; the former retained the radionuclide longer than any other tissue. Pretreatment of trout with the potent antiandrogen cyproterone acetate significantly reduced radioactivity in plasma of both sexes, in testes but not ovaries, and possibly in the liver of the male. No effect due to the androgen antagonist was apparent in kidney, spleen, gall bladder, brain, pituitary, or epaxial muscle. High levels of radioactivity found in the bile indicates that the trout were using a hepatic route for clearance of the exogenous steroid.

Little is known about the function of various organs or tissues of fishes in accumulation and retention of sex hormones. The available information concerning sex endocrine targets is primarily indirect and concerns observations of tissue responses to hormone treatment. For example, McBride and van Overbeeke (1971) found that 11-ketotestosterone or 17α-methyltestosterone treated orchiectomized adult sockeye salmon (*Oncorhynchus nerka*) had a significant increase in epidermis thickness, a marked atrophy of the stomach, and a degeneration of the liver and kidney. They reported similar responses in ovariectomized fish treated with estradiol or estradiol cyprionate with the liver appearing hyperactive.

This report deals with an attempt to establish which tissues or organs can accumulate androgen and to determine possible rates of uptake and loss from these structures in rainbow trout (*Salmo gairdneri*). The antiandrogen cyproterone acetate (CA) (1,2α-methylene-6-chloro-Δ4,6-pregnadiene-17α-ol-3,20-dione-17α-acetate) was used to help illustrate areas where testosterone may be acting as an androgen or in some other capacity and to determine its effect in fish.

The effects of CA have heretofore never been determined in fishes. The action of this synthetic steroid in laboratory mammals was reviewed by Neumann and Elger (1966), Neumann *et al.* (1967a,b), and Elger *et al.* (1967a). CA, a highly progestational compound (Hamada *et al.*, 1963), inhibits the sexual actions of endogenous or exogenous testosterone. Male fetuses of pregnant rats, mice, or rabbits treated with the antiandrogen are feminized, and female secondary and accessory sex structures develop (Elger, 1966; Elger and Neumann, 1966; Elger *et al.*, 1967b; Hamada *et al.*, 1963; Junkmann and Neumann, 1964; Neumann *et al.*, 1966a,

[1] Present address: Division of Forestry and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.

Reprinted from COPEIA, 1972, No. 4, December 29
pp. 865–868
Made in United States of America

EVALUATION OF DIEL VARIATION IN ANDROGEN LEVELS OF RAINBOW TROUT, *SALMO GAIRDNERI*.—Little is known about reproductive endocrinology in teleostean fishes. We, therefore, designed an experiment to determine the possibility of diel variation in androgen levels of male rainbow trout (*Salmo gairdneri*). The circulating levels of male sex hormone were also compared with gonadal development in both sexes, and the androgen response to the stress of blood sampling by cardiac puncture was evaluated.

A diurnal rhythm in plasma glucocorticoid levels exists in the channel catfish (*Ictalurus punctatus*) (Boehlke et al., 1966). Although definitive proof is lacking, diel periodicity in plasma testosterone possibly occurs in the skate *Raja radiata* (Fletcher et al., 1969). Androgen assay procedures used in prior studies, however, required large, often pooled, volumes of plasma, and thus correspondingly large fish. These methods also had laborious techniques not permitting elaborate experimental design. The competitive protein-binding assay, suggested by Murphy (1968) made it feasible to determine androgen levels in individual small fish.

Methods.—Two-year old fall spawning rainbow trout (300-400 mm total length) were kept in a raceway at the Bellvue Experimental Fish Hatchery, Bellvue, Colorado.

The trout were bled via cardiac puncture with a heparinized syringe during their normal spawning season (30 and 31 October 1970). Blood samples were taken from male fish at 1800, 2400, 0600, and 1200 hours during a 24-hour period. Females were sampled only at 1800 hours. In the male trout, called terminal fish, 10 different fish were bled each time at 2400, 0600, and 1200 hours; these 30 individuals were different than the original 28 fish sampled at 1800 hours. In the repeat group, blood (1.0–1.5 ml) was obtained 4 times from the same 20 males, once at each of the sampling periods. Mortality in the repeat group resulted in a decline in number of samples obtained at each successive sampling. Fin clips were used to identify individual repeats, and this group was returned to a section of the same raceway between sampling times.

Sex and maturity of all fish were confirmed at autopsy. Intact fish were weighed and gonado-somatic indices (GSI) determined.

The competitive protein-binding assay as employed in mammals primarily measures testosterone; to a lesser extent a few other androgens and estrogens may be assayed (Horton et al., 1967). Estrogen levels, however, are of negligible magnitude in comparison with androgen concentrations reported here and are too low to influence the androgen determinations. Plasma (0.1 ml), to which a drop of 0.4 N NaOH was added, was double extracted with 2 ml spectrophotometric grade $ChCl_3$. A portion (40%)

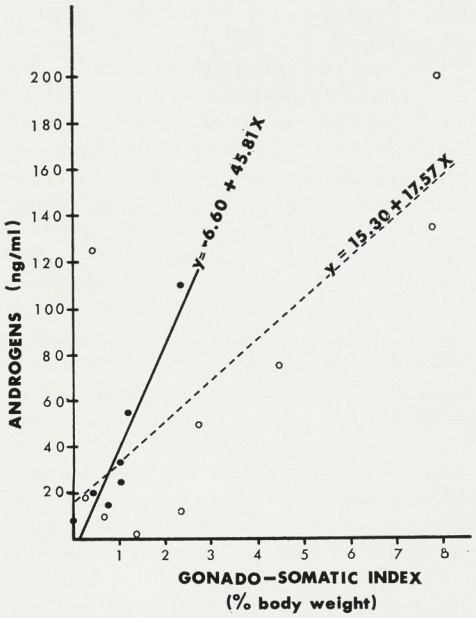

Fig. 1. Relationship of androgen level (ng/ml plasma) and gonado-somatic index in male and female rainbow trout (males—dots and solid line; females—circles and dashed line).

of the combined extracts in 15 × 85 mm culture tubes was evaporated to dryness at 45 C under nitrogen. The residue was shaken with 1 ml ^{3}H-testosterone-saturated sex hormone binding globulin (10 ng containing 5×10^6 dpm 1,2 ^{3}H-testosterone and 0.3 ml human late pregnancy [third trimester] plasma made up to 100 ml with deionized water). Tubes were shaken gently, placed in a 45 C water bath for 5 minutes, transferred to an ice-water bath for 10 minutes, and shaken gently while in the ice bath. Thereafter, 80 mg washed Florisil (magnesium silicate, 60–100 mesh, Sigma Chemical Co.) was added to a tube which was immediately shaken for 30 seconds and returned to the ice bath for 3 minutes. Immediately following, 0.5 ml supernatant was placed in a liquid scintillation vial to be counted. Intervals between tubes were constant. A standard curve of percentage counts displaced by 0, 1.0, 2.0, 3.0, and 4.0 ng authentic testosterone as determined by the competitive protein binding procedure served for estimation of androgen levels in the samples. Randomization of our samples insured against bias or experimental error. Results of this method are replicable.

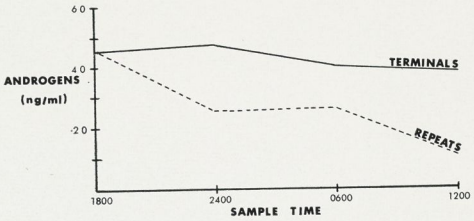

Fig. 2. Androgen levels (ng/ml plasma) of terminal and repeat rainbow trout.

Results.—There is a strong correlation between GSI and plasma androgen level in both male and female trout. Data and simple linear regression lines are shown in Figure 1. Regression analysis for males shows that GSI and androgen level are related ($F = 39.8$, $P < 0.005$; $R^2 = 0.89$). The simple linear regression for females is also significant ($F = 8.95$, $P < 0.025$; $R^2 = 0.56$). The slopes of these two lines are significantly different ($F = 5.8$, $P < 0.025$, $R^2 = 0.56$) from each other.

There was no significant difference in androgen level between terminal fish sacri-

Table 1. Androgen Levels (ng/ml) in Plasma of Rainbow Trout and Statistical Analysis.

Treatment and Time	n	Mean	Range	SD
Terminal males				
1800	28	45.9	8–110	26.6
2400	10	46.8	20–100	24.8
0600	10	39.7	18–65	13.3
1200	10	37.1	20–85	19.1
Repeat males				
1800	20	45.8	15–100	21.1
2400	17	26.2	12–62	13.6
0600	11	33.7	13–100	26.2
1200	9	15.0	5–48	13.5
Repeat males (survivors)[1]				
1800	9	41.4	15–78	19.4
2400	9	21.2	12–45	10.7
0600	9	37.7	14–100	27.6
1200	9	15.0	5–48	13.5
Females				
1800	11	78.3	2–200	66.4

[1] Includes only the 9 individuals which survived to be bled four times.

Reprinted from *J. Fish Biol.* (1973) **5**, 227–230

Plasma oestrogen levels in rainbow trout *Salmo gairdneri* Richardson

CARL B. SCHRECK*
Colorado Cooperative Fishery Unit, Colorado State University, Fort Collins, Colorado 80521, U.S.A.

ROBERT T. LACKEY
Division of Forestry and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, U.S.A.

AND

M. LLOYD HOPWOOD
Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado 80521, U.S.A.

(*Received 27 June 1972*)

Levels of oestrogens in plasma of mature fall-spawning rainbow trout *Salmo gairdneri* were determined using a radio-immunoassay. No significant difference was found in oestrogen concentration between the sexes (male $\bar{x}=2{\cdot}5$ ng/ml; female $\bar{x}=4{\cdot}4$ ng/ml); between individual variability was great. Four blood samplings over a 24-h period via cardiac puncture of males revealed no diel variation or change in estrogen levels due to the stress of bleedings. Although no gonado-somatic index-estrogen relationship could be demonstrated for either sex, there was correlation between oestrogen and androgen levels in the female.

I. INTRODUCTION

There are few studies concerning levels of circulating oestrogens in fishes. Eleftheriou *et al.* (1966) reported channel catfish *Ictalurus punctatus* have an increase in free plasma oestrogens with an increase in ovarian weight. They also observed an increase in oestrogens from prespawning to the end of the spawning period. Cedard *et al.* (1961) demonstrated fluctuations in plasma oestrogens in Atlantic salmon *Salmo salar* of both sexes during maturation with levels highest in ripe fish and lowest in the post-spawned group. The levels of female sex hormone in rainbow trout determined on relatively large numbers of individuals under treatment conditions are reported. The use of a recent solid-phase radioimmunoassay (Abraham, 1969) allows quantification of oestrogens in minute plasma volumes, thus eliminating possible error of pooled samples. This technique allowed us to investigate the possibility of diel variation. The effects of stress due to repeated cardiac puncture blood sampling were also evaluated for the male trout. The relationship of gonadal development to endogenous plasma oestrogens was determined for both sexes. The radioimmunoassay for oestrogens has been shown to measure oestradiol-17β with oestrone and oestriol

*Present address: Division of Forestry and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, U.S.A.

implicated to a lesser extent (Abraham, 1969). Testes and ovaries of rainbow trout reportedly contain oestradiol-17β, oestriol, and 16-epioestriol (Galzigna, 1961). The steroids were quantified as oestrogens.

II. METHODS AND MATERIALS

EXPERIMENTAL DESIGN

Two-year-old fall-spawning hatchery rainbow trout (300–400 mm) were bled via cardiac puncture during spawning season (October 1970). Blood (1·0–1·5 ml) was collected from males at 1800, 2400, 0600 and 1200 h during a 24-h period. Females were sampled only at 1800 h. Males were divided into two groups, referred to as ' terminals ' and ' repeats '. Terminals consisted of 28 fish sampled at 1800 h and 10 different fish sampled once and then killed at each of the other three times, respectively. Only fish from which adequate blood was obtained were used in the analysis (Table I). Terminal fish were not sampled more than once. The 20 repeat fish were bled once at each of the sampling times; i.e. each fish was bled four times, once every 6 h, if they survived the study period. Plasma samples were stored at −15° C. Fish were internally sexed and gonado-somatic indices (GSI) determined. The experimental design is also discussed by Schreck *et al.* (1972*b*).

TABLE I. Oestrogen levels (ng/ml) in plasma of rainbow trout and statistical analysis

Treatment and time	*n*	Mean	Range	S.D.
Terminal males				
1800	24	2·5	1·0–5·6	1·2
2400	9	2·0	1·1–3·1	0·7
0600	10	3·3	1·5–5·9	1·2
1200	9	2·8	1·5–4·1	0·8
Repeat males				
1800	17	2·4	1·0–4·3	0·9
2400	17	2·0	1·1–4·4	0·8
0600	11	2·4	1·1–3·6	0·9
1200	9	1·8	0·7–3·8	1·0
Repeat males (survivors)*				
1800	9	2·6	1·3–4·3	1·0
2400	9	2·2	1·1–4·4	1·0
0600	9	2·4	1·1–3·6	0·9
1200	9	1·8	0·7–3·8	1·0
Females				
1800	9	4·4	1·8–5·0	2·0

*Includes only the nine individuals which survived to be bled four times.

ASSAY

The oestrogen assay was essentially as reported by Abraham (1969) except that an anti-oestradoil-17β antibody prepared by Ekre & Foote (1971) was used at a dilution of 1 : 1000 to coat the plastic tubes. The specificity of the assay for several steroids was tested at five different levels, including physiological ranges. The antibody was unreactive to androgens (androstenedione, testosterone), progestins (progesterone, 17α-hydroxyprogesterone), and corticoids (cortisol, corticosterone) but bound oestradiol-17β and oestrone equally and oestriol slightly. The sensitivity of the method is 12–15 pg for estradiol-17β. Authentic oestradiol-17β at five different concentrations ranging from 12·5–400 pg were assayed 32

Reprinted from PROC. SOC. EXP. BIOL. AND MED. Volume 140, Number 3 July 1972

PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **140**, 1009–1011 (1972)

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

CARL B. SCHRECK, STEPHEN A. FLICKINGER, AND M. L. HOPWOOD

Colorado Cooperative Fishery Unit and Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado 80521

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 × 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

[1] Supported in part from the Commercial Fisheries Research and Development Act (P.L. 88-309) Project 6-11-D and cooperatively by the Bureau of Sport Fisheries and Wildlife, Colorado State University, and Colorado Division of Game, Fish and Parks and Colorado Experiment Station Project 57.

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

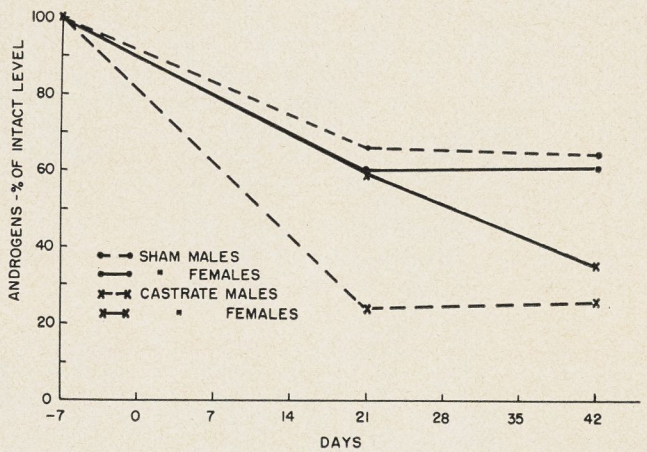

FIG. 1. Androgens in castrate and sham-castrate trout. Surgery was performed on day 0.

Reprinted from PROC. SOC. EXP. BIOL. AND MED. Volume 143, Number 1 May 1973

PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 143, 189–191 (1973)

Binding of Cyproterone Acetate to Plasma Proteins[1] (37283)

PAUL H. SAMMELWITZ,[2] CARL B. SCHRECK,[2] AND M. L. HOPWOOD

Department of Animal Science and Agricultural Biochemistry, University of Delaware, Newark, Delaware 19711; Department of Fisheries and Wildlife Science, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061; and Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado 80521

Cyproterone acetate (CA) (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3,20-dione-17α-acetate) is a potent antiandrogen with high progestational activity (1). Even when administered concurrently with androgens, CA generally reduces the size of male accessory sex organs. Its effect is on target tissues responsive to androgens (2). In the rat CA reduces the retention of testosterone in seminal vesicles and glans penis but not in brain, muscle or plasma (3). In brain, however, Stern and Eisenfeld (4) found the uptake of testosterone to be antagonized by the nonesterified form, cyproterone. Neumann, Elger and VonBerswordt-Wallrabe (5) suggested that CA attaches to androgen receptor sites, preventing binding of endogenous androgens to target tissues. In support, competition between testosterone and cyproterone has been demonstrated for binding sites on seminal vesicular macromolecules *in vitro* and *in vivo* (6).

Several plasma globulins possess specific binding affinities for steroid hormones. Sex hormone-binding globulin (SHBG) in human late pregnancy plasma (7) has been shown to specifically bind C_{19} steroids and to react slightly with C_{18} steroids but not with C_{21} steroids (8). Corticosteroid-binding globulin (CBG) in dog plasma (9) is specific for C_{21} steroids. We tested the possibility of receptor sites for CA, a C_{21} steroid derivative, on the two plasma globulins. Consideration is also given to competition between CA and representatives of other classes of steroids for binding sites on SHBG and CBG.

Materials and Methods. To test the displacement of ^{3}H-testosterone from SHBG 1,2-^{3}H-testosterone[3] at 1.3×10^7 dpm was added to 100 ml of 0.3% pooled human late pregnancy plasma as outlined by Murphy (9) and displaced with authentic testosterone, estradiol-17β (E_2) or CA[4] at concentrations of 0, 1, 2, 3 or 4 ng/tube. The assays employing 1 ml of the SHBG preparation were run in duplicate or triplicate. Florisil (magnesium silicate, Sigma Chemical Co.) was used to separate free from protein-bound steroid according to Neill *et al.* (10).

To determine the displacement of ^{3}H-estradiol-17β (^{3}H-E_2) from SHBG 6, 7-^{3}H-E_2 at 1.7×10^7 dpm was added to 100 ml of 0.3% SHBG. The labeled steroid was displaced with authentic E_2, testosterone or CA at 0, 1, 2, 3 or 4 ng/tube. Assays using 1 ml of the SHBG preparation were run in duplicate as above.

Displacement of ^{3}H-corticosterone from CBG was tested by adding 1,2-^{3}H-corticosterone at 11×10^6 dpm to 100 ml of 2.5% dog serum (9). The labeled steroid was

[1] Supported in part from Colorado Experiment Station Project 57, Commercial Fisheries Research and Development Act (P. L. 88-309) Project 6-11-D, and cooperatively by the Bureau of Sport Fisheries and Wildlife, Colorado Division of Game, Fish, and Parks, and Colorado State University.

[2] This work was carried out while P. H. S. was in the Department of Physiology and Biophysics, and C. B. S. was in the Colorado Cooperative Fishery Unit, Colorado State University, Fort Collins, CO.

[3] All radioactive steroids from Amersham/Searle Corp.

[4] We thank Dr. F. Neumann, Schering AG., Berlin, Germany, and Dr. R. O. Neri, Schering Corp., Bloomfield, NJ, for the cyproterone acetate.

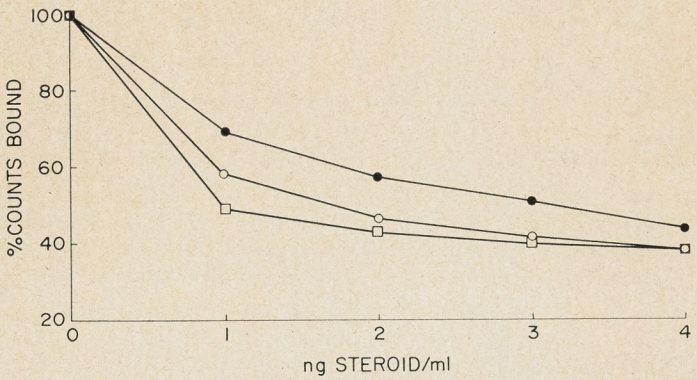

FIG. 1. Displacement of ^{3}H-testosterone from sex hormone-binding globulin (SHBG) by estradiol-17β (●), cyproterone acetate (CA) (□), and testosterone (○).

displaced with authentic progesterone, corticosterone or CA at 0, 0.5, 1, 2, 3 or 4 ng/tube as above.

Results and Discussion. CA was as effective as testosterone in displacing ^{3}H-testosterone from SHBG and E_2 was less effective (Fig. 1). Cyproterone, however, reportedly has less than 5% the binding affinity of testosterone for a testosterone-binding globulin preparation (11). The close similarity between the CA and testosterone displacement curves plus the fact that E_2 displaces relatively less bound testosterone suggests that there is a greater similarity between binding sites for testosterone and CA than between testosterone and E_2.

CA was less capable of displacing ^{3}H-E_2 from SHBG than testosterone or E_2 (Fig. 2). Displacements by the latter two steroids were very similar, indicating that similar binding sites may be shared by E_2 and testosterone. Generally, displacement of ^{3}H-E_2 from the protein was less complete than displacement of ^{3}H-testosterone (Fig. 1). It has been shown (8) that E_2 will maximally displace 20% of labeled testosterone from SHBG. CA will displace over 65% of labeled testosterone from SHBG but a minimal amount of ^{3}H-E_2. This differential displacement suggests that E_2 occupies binding sites that differ from those of CA. Our explanation of this enigma is that testosterone and E_2 occupy similar but not identical sites on SHBG whereas CA and testosterone interact on the same site.

CA (a C_{21} steroid), unlike progesterone or corticosterone, does not displace ^{3}H-corticosterone (Table I). None of the C_{21}

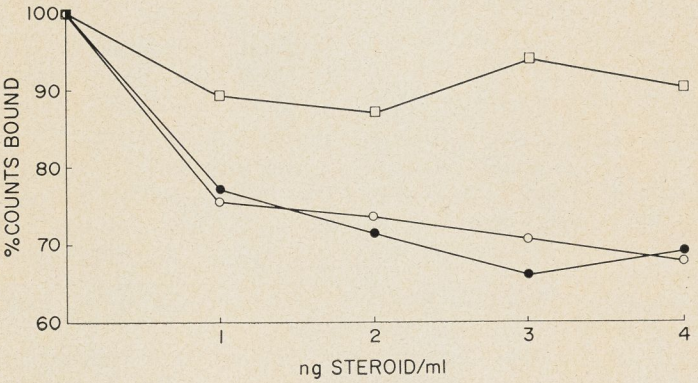

FIG. 2. Displacement of ^{3}H-estradiol-17β from sex hormone-binding globulin (SHBG) by estradiol-17β (●), cyproterone acetate (CA) (□), and testosterone (○).

COLLEGE OF AGRICULTURE AND LIFE SCIENCES

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061

DIVISION OF FORESTRY AND WILDLIFE RESOURCES (703) 951-5481

Department of Fisheries and Wildlife Sciences

February 19, 1974

Dr. Robert J. Behnke
Colorado Cooperative Fishery Unit
Colorado State University
Fort Collins, Colorado 80521

Dear Bob:

It is high time that I write, and your letter has given me a good reason to do so today. There isn't much that I can say concerning the Bureau's action; it is the type of thing, however, that I would expect from them. There are a lot of people here and in D.C. that are very dissatisfied with the Research Division. It seems to me that you made the choice that you had to make.

Concerning consulting prospects, I have some contacts at the N.P.S. Denver Service Center. I did some work for them in December on aquatic resources in Charleston Harbor. I will contact them and mention that you are available if you wish. I guess you know that John Windell has a consulting group at C.U.

The Head of the Department of Fisheries and Wildlife is still available here; this probably wouldn't interest you though. We do have the possibility of employing visiting scholars for a quarter or two. Would you be interested in such a position if I could swing it with John Hosner? You could, say, study native brook trout in the Appalachians, Great Smoky ~~Natural~~ National Park in particular, and teach one course or seminar on systematics and evolution. I really think that employment here for a quarter or two is a distinct possibility.

I am enclosing a couple of papers that may be of interest. I am also enclosing a set of my reprints. I have a couple of other papers coming out shortly and a small book with Victor deVlaming and Bob Harrington called Sex Control in Fishes that will be published by Sea Grant. I will send you copies when available. I also hope that you can make use of the updated handouts from my courses.

We have a hatchery nearby that raises rainbow trout. About one trout out of 50,000 is baby blue in color. Although the blue fish can be raised to a size of about 12 inches, they are much more delicate than the wild form and we are told that they don't mature sexually. Are you familiar with this condition? Is there any literature on them? Should we bury an aguabonita in the Owens Valley for Miller to find?

Please let me know your thoughts on the above matters and if I can help you in any way.

Best regards,

Carl

Carl B. Schreck
Assistant Professor,
Fisheries Science

CBS:cwl

Enclosures

P.S. Please give my best regards to your family. We really enjoyed the picture Cynthia & Bobby you sent at Christmas. Boy have they grown!

We still have our eyes set on returning to the West someday. Please let me know if you hear of anything.