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Attempts to Inhibit Testicular Growth in Rainbow Trout with Antiandrogens (Cyproterone, Cyproterone Acetate, Oxymetholone) and Busulfan Given during the Period of Spermatogenesis

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We tried to inhibit spermatogenesis in adult male rainbow trout by giving them a daily diet (1% of body wt) containing antiandrogens—cyproterone, cyproterone acetate (Schering), oxymetholone (Syntex)—at dose of 20 or 40 mg/kg in the food, or by injecting an antimitotic agent, busulfan (Misulban, Techni-pharma), at doses of 10 mg/kg of body weight twice a month for 153 or 162 days, over the main period of testicular activity. When the cyproterone acetate and oxymetholone treatments were started before initiation of spermatogenesis, there was no testicular growth, but when spermatogenesis had already begun in June, the inhibitory effect of these drugs was limited; cyproterone acetate in that case had no effect at all. A limited number of spermatogonia were present in the undeveloped testis, suggesting that the effect could be reversible. GTH secretion was not suppressed, indicating that the drug had a direct effect on the gonads. During the experiment, body weight increased by 18% in the control group. In the groups given inhibitory treatments, body weight decreased slightly (6%) with cyproterone acetate but was markedly reduced (10%) after the oxymetholone treatment. Large-scale experimentation is necessary to test the effect of these drugs on growth. In conclusion, it appears that only cyproterone acetate, preventing male sexual maturation, is of interest in fish culture. It may also prove to be a useful tool for laboratory experimentation.

Techniques of fish gonad sterilization are useful in both the practice of fish culture and in fish management, for instance, when introducing new species in rivers or lakes. Some methods are based on a genetic approach (Chevassus *et al.*, 1979) while others use steroid administration. The latter technique has been carried out in juveniles at the time of sex differentiation (Jalabert *et al.*, 1975; Yamazaki, 1976; Johnstone *et al.*, 1978; Goetz *et al.*, 1979) or in adults during gametogenesis (Billard *et al.*, 1981). The use of steroids, however, may face problems of legal limitation, and so other drugs need investigation. The present paper reports the effects of three antiandrogens (cyproterone, cyproterone acetate, and oxymetholone) and one antimitotic agent (busulfan) on testicular development and circulating gonadotropin (t-GTH) in adult

rainbow trout males. Antiandrogen has been reported to reversibly inhibit spermatogenesis in mammals (Steinbeck *et al.*, 1971; Morse *et al.*, 1973) and to have behavioral effects on fish (Rouse *et al.*, 1977), as well as inhibitory effects in secondary sex characters (Hamaguchi, 1978). Busulfan, an alkylating substance with antimitotic activity, is reported to sterilize male rats when administered *in utero* (Hemsworth and Jackson, 1962; Gillet and Laporte, 1973) and to limit fertility in adults (Jackson *et al.*, 1959).

MATERIAL AND METHODS

Our experiments were conducted in 1975 and 1976. Two-year-old rainbow trout males weighing about 200 g and originating from the Forgeot fish farm were brought to our laboratory in March and kept in recycled water in our experimental facilities. They were still at the running stage and easy to sex. We fed them

1% of their body weight daily in regular fish pellets (Trouw) until the experiment began on June 15, 1975 and on May 10, 1976. At this time some males were killed for removal of gonads and histological determination of the stage of spermatogenesis. Groups of 11 to 15 fish were fed the regular pellets above (controls) or those containing 20 or 40 mg/kg of an antiandrogen: cyproterone or cyproterone acetate (CA) (Schering AG-D1, Berlin 65, Postfach 650311) or oxymetholone (Syntex, Hillview, Palo Alto, Calif.) (Table 1). The drugs were first dissolved in oil which was mixed with wheat, fishmeal, vitamins, and minerals to make the pellets. These were fed daily at 1% of body weight. The antimetabolic compound, busulfan (Misulban, Techni-pharma, BP 159, Monaco), was injected intraperitoneally at a dose of 10 mg/kg of body weight/injection with 2 injections/month. The fish were weighed periodically, and their blood was sampled for trout gonadotropin (t-GTH) (glycoprotein) which was measured by radioimmunoassay in the same manner as for carp (Breton *et al.*, 1971). The experiments ended on November 24, 1976. All the fish were then checked for signs of sperm release and killed after they had been starved for 3 days; the body, gonads and liver were weighed to calculate the gonadosomatic index (GSI) and the hepatosomatic index (HSI). The testes were fixed in Bouin-Hollande and the 7- μ m sections stained with hematoxylin for histological examination.

Variance analysis was used to statistically compare the results after log transformation for the GSI. Values on figures and table are given $\pm$ SD.

RESULTS

Gonadal Changes

During the 1976 experiment, which started in May the testes were still in the resting stage, only type-A spermatogonia (G_A) were found in the lobule with occasional spermatozoon resorption. Cyproterone acetate and oxymetholone entirely prevented gonadal development, and at the end of the treatment the GSI was significantly lower ($P < 0.005$) than in the controls (Table 1). Some isolated G_A 's were occasionally present in the undeveloped testis, but no other germ cells were found.

The 1975 experiment started in June after some spermatogenetic activity had begun in the testes: type-B spermatogonia (G_B) and some spermatocytes I. Cyproterone had only a limited effect: the gonads in 2 males remained at the resting stage, while 3 males

showed signs of spermatogenetic activity and 2 others were fully mature (spermiating). Cyproterone acetate had no effect and all the males were in the running stage. Busulfan had irregular effects: 2 male gonads were still regressed and 4 were in full spermiation.

Histological examination showed that even in the entirely regressed gonad, some G_A 's were present. The structure of the testis was altered, however: the lobular structure was hardly visible, the gonadal wall was very thick and there was considerable connective tissue.

GTH Changes

In the 1975 experiment, the mean GTH level was not much different in the controls and in the cyproterone-treated group but was higher in the cyproterone acetate-treated group (Fig. 1). In cyproterone-treated males which had reached maturity, t-GTH was higher than in males with regressed testis. Plasma GTH level was even higher in the mature cyproterone acetate-treated males than in the mature controls (Fig. 1). It was not altered by the busulfan treatment (Fig. 2).

The results on the 1976 experiment were quite different (Fig. 3): there was no elevation in plasma GTH in the treated fish; the rise observed in the controls toward the end of the experiment occurred later in the cyproterone acetate-treated group and not at all in the fish given oxymetholone.

Changes in Growth

No treatment side effects were seen to affect the HSI, but the fish lost weight with the oxymetholone treatment (10%) (Fig. 4). The overall weight gain during the experiment was low in all the other groups, even in the controls (Table 1).

DISCUSSION

The effects of antiandrogen treatment were not the same in the 1975 and 1976 experiments. One of the major differences

TABLE 1
EFFECTS OF SOME ANTIANDROGENS AND BUSULFAN (ANTIMITOTIC AGENT) ON RAINBOW TROUT SPERMATOGENESIS^a

Treatment	Dose in diet (mg kg ⁻¹)	No. of fish start end	Final body weight (g)	Body weight (g) ^b	GSI (%)	HSI (%)	Regressed males (%)	Spermiating males (%)
1975								
Controls	0	15	264 ± 71	+47	4.9 ± 1.8	1.5 ± 0.37	0	100
Cyproterone	20	15	292 ± 26	+71	5.8 ± 1.1	1.2 ± 0.24	0	100
Cyproterone	40	15	280 ± 10	+75	2.4 ± 4.3	1.2 ± 0.1	30	42
Busulfan	10 ^c	15	253 ± 77	+60	4.7 ± 2.3	2.1 ± 0.3	33	66
1976								
Controls	0	13	231 ± 47	+5	2.6 ± 0.11	2.2 ± 0.39	0	100
Cyproterone acetate	20	11	231 ± 52	+14	0.6 ± 0.02*	2.2 ± 0.1	100	0
Oxymetholone	5	11	198 ± 32	-19	0.4 ± 0.02*	2.1 ± 0.2	100	0

^a Duration of treatment was 153 days in 1975 (15/06 to 24/11) and 162 days in 1976 (10/05 to 29/10).

^b Changes in body weight during the experiment.

^c Dose per injection: 10 mg/kg of body weight, given intraperitoneally twice a month.

* $P < 0.005$ as compared to control.

FIG. 1. Changes in immunoreactive t-GTH in plasma of male rainbow trout following treatment with cyproterone (Cyp) and cyproterone acetate (CA) added to the diet (respectively, 40 and 20 mg/kg of pellet). Control males received normal diet. Treatment started on May 10, 1976 when gonads were still in a resting stage (NS, nonsignificant; there was no significant difference between the three groups). □, Control; ○, Cyp; ▲, CA.

was the stage of spermatogenesis when the treatment started. It therefore seems that testicular development can be inhibited by antiandrogens (cyproterone, cyproterone acetate, and oxymetholone) only when the treatment begins before spermatogenesis has started. After that, the results are heterogeneous and gonadal development is prevented only in a limited number of cases. Murphy (1980) also observed that

FIG. 2. Changes in t-GTH in plasma of male rainbow trout receiving one intraperitoneal injection twice a month of Busulfan (10 mg/kg body wt/injection) during 153 days. Treatment started on May 10, 1976 (Day 0) before spermatogenesis had started. Control group is the same as in Fig. 1. There is no difference between the 2 groups. □, Control; ●, busulfan.

FIG. 3. Changes in immunoreactive t-GTH in plasma of rainbow trout males receiving oxymetholone (OX) and cyproterone acetate (CA) added to the diet (5 and 20 mg/kg of food, respectively). When the treatment started (15/6/1975) spermatogenesis had already begun. Control group received normal pelleted food. □, Control; △, OX; ■, CA.

cyproterone acetate given to Atlantic salmon parr at the end on spermatogenesis had no effect on testis weight. Rouse *et al.* (1977), however, reported an inhibition of the formation of spermatids and spermatozoa in stickleback after cyproterone acetate treatment. The subsistence of G_A 's in the undeveloped testis indicates that the effect of the treatment may be reversible. The consequences of antiandrogenic treatment on circulating t-GTH are not clear. When gonadal development was prevented (1976 experiment), the GTH levels were not significantly different from the controls, suggesting a direct effect on the gonad rather than on the central nervous system. But when the treatment did not prevent testicular development, the mature treated

FIG. 4. Changes in body weight (BW) in the oxymetholone (OX) and cyproterone acetate (CA) treated groups (1975 experiment). □, Control; ▲, OX; ○, CA.

males had a higher plasma GTH concentration than the controls at the same stage of maturity. A direct effect on the gonads is also suggested by the results of Schreck (1973) who showed a blockage of the uptake of tritiated testosterone on trout testis by cyproterone acetate and Murphy (1980) who reported a significant decrease of plasma 11 ketotestosterone after cyproterone acetate treatment in salmon parr. Gonadal negative feedback (Billard *et al.*, 1977) at the hypothalamo-pituitary level might be limited by the antiandrogenic action of the drug, as shown in rats by Viguier-Martinez (1976), but a similar action did not occur in the testis since spermatogenesis was normal. In addition, the progestational side effect of cyproterone acetate, which in mammals reduces GTH release (Steinbeck *et al.*, 1971), did not appear in trout, as shown in this experiment. It has already been reported that progestogens tend to stimulate GTH secretion (Billard *et al.*, 1981). The doses of cyproterone and cyproterone acetate used in this experiment were relatively low (about 0.2 and 0.4 mg/kg of body wt/day); higher doses might have been more efficient in maturing males. In rats, reduction in fertility is related to the dose, and a reduction in testis weight is observed after daily treatment with 20 mg of cyproterone acetate for 5 weeks (Back *et al.*, 1977).

The other antiandrogen, oxymetholone, which was tested only in the 1976 experiments, was as potent as cyproterone acetate in inhibiting gonadal growth but it decreased body weight during the experiment, thus limiting its practical use in fish culture.

The antimetabolic agent, busulfan, had only a limited effect: only 2 out of 6 fish were sterilized with the dose used (0.7 mg/day). This is quite different from the results reported in mammals in which a single dose (about 6 to 16 mg/kg of body wt) of busulfan or other sulfonic ester induces a noticeable reduction in spermatogenesis by inhibiting mitosis in the spermatogonia or by damag-

ing the postmeiotic stages (Gilmore, 1973) in various species: rat (Partington *et al.*, 1964), rabbit (Fox *et al.*, 1963), mouse and hamster (De Rooij and Kramer, 1970). Therefore, this compound is not very promising as an antifertility agent for adult fish.

In conclusion, fish spermatogenesis may be inhibited by using antiandrogens such as cyproterone acetate and oxymetholone added to the diet, provided that the treatment is started before spermatogenesis is initiated. Cyproterone acetate seems more suitable than oxymetholone because the fish do not lose weight during the treatment. The effects are possibly reversible since germ cells are still present in the testis after more than 150 days of treatment; however, this point remains to be demonstrated because the structure of the testes was considerably modified. It would also be interesting to see if the treatment is necessary throughout the whole period of spermatogenesis rather than just the start. In addition, this would indicate if the antiandrogens can be withdrawn for some weeks or months from marketing to allow flesh residues to disappear without a corresponding testicular recrudescence. This is not likely to happen in the winter where the environmental conditions are not appropriate for recrudescence of spermatogenesis but this may be possible in summer and early autumn.

Because of this possibility of regeneration these drugs could not be used for sterilizing the gonads definitively in foreign species released in the wild. Cyproterone acetate might be useful in intensive fish farming but since growth rate was somewhat lower than in the controls, extensive experimentation is necessary before definitive conclusions can be reached on the influence on growth.

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