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Stimulation of Gonadotropin Secretion after Castration in Rainbow Trout

The effects of bilateral castration on plasma gonadotropin (t-GtH), as measured by radioimmunoassay, were investigated in male rainbow trout at several stages in the reproductive cycle: at the start of spermatogenesis at the end of May, after its completion in September, and before and after the beginning of spermiation in October and December. Castration resulted in increased level of circulating immunoreactive t-GtH but the response varied with the season. The rise after castration was four times the initial value in May and September, and increased twofold in October and sevenfold in December. The duration of the response following castration was 4 months in May, 2 months in September, and less than 1 month in October and December.

Indirect evidence for gonadal regulation of the gonadotropic function in teleost fish was given by Atz (1955) and McBride and Van Overbeeke (1969) who showed changes in the gonadotropic cells of the pituitary after physiological or surgical castration. More direct evidence of gonadal feedback was the short-term elevation of gonadotropin concentration in response to castration of trout (Billard *et al.*, 1976).

In the present experiments bilateral castration was performed in rainbow trout at various points in the spermatogenetic cycle: at the end of May when type A spermatogonia (spermatogonia not included in cysts) increase in number in the testis, in mid-September when spermatogenesis is ended, and in October and December before and after the peak of secretion which induces spermiation (Breton *et al.*, 1975). The plasma gonadotropin concentrations in all groups were followed until the following January.

MATERIALS AND METHODS

Males weighing between 150 and 200 g, taken after their first reproductive cycle, were kept in the laboratory under a natural photoperiod (see Fig. 1). Rearing temperature is shown in Fig. 1. At the end of May and September, and in mid-October and mid-December, 10 to 15 fishes were castrated whilst anesthetized by recirculating a thermo-regulated solution of MS 222 (Sandoz Ltd) over the gills. The testis and vas deferens were removed through a 3- to 4-cm ventral incision anterior to the abdominal fins. After

operation 100,000 units of penicillin (Specia) were poured into the body cavity and the wound was sutured with tressed nylon (Lyganyl, No. 3). The intact fish were kept under the same conditions as a control group. Blood samples were taken by heart puncture from control and experimental males before operation and at various intervals thereafter (once a week to once or twice a month). Samples were kept on chipped ice prior to centrifugation at 3000 rpm for 20 min. Plasma was stored at -20° until analysis.

The radioimmunoassay procedures for trout gonadotropin (t-GTH) were similar to the ones used for carp (Breton *et al.*, 1971) with use of highly purified t-GTH (Breton *et al.*, 1976) preparation either for iodination or as standard. The antibody was raised in guinea pig using the above highly purified t-GTH (0.5×10^{-5} , final concentration in a 500- μ l volume assay). For all samples duplicate determinations were made, each on 50 μ l of 1:3 diluted sample.

At various points in the reproductive cycle, testes of intact males were weighed and fixed in Bouin-Hollande solution in order to calculate the gonadosomatic index (GSI) and check the stage of spermatogenesis by histological examination.

Results obtained from castrated and intact groups were compared by *t* tests and analysis of variance.

RESULTS AND DISCUSSION

In control fish the level of plasma t-GTH did not vary much from May to November but decreased in December and January despite the fact that spermatogenesis was in progress in July and August (when GSI increased sharply), and was completed by the end of September (Fig. 1). Spermiation started at the beginning of November and most of the males were in a running stage at

FIG. 1. Bottom graph, circulating gonadotropin in control and in castrated male rainbow trout at different stages of the reproductive cycle. Middle, GSI variation in the same population of males during the same period. Top, evolution of the environmental factors: natural photoperiod and rearing temperature (week average). Data are given with the standard deviation. ☆, $P < 0.05$; ☆☆, $P < 0.01$; ☆☆☆, $P < 0.001$.

the end of November. Between October and December GSI decreased significantly ($P < 0.01$). This decrease of GSI in December may be due to the sperm release but remains to be explained in October and November.

The effects of bilateral castration at various points in the reproductive cycle of male rainbow trout were quite variable both in intensity and duration of the response. In animals castrated in June, t-GTH increased rapidly and significantly ($P < 0.01$) after

castration and reached its maximum values within 10 days as previously reported (Billard *et al.*, 1975). This maximum value was maintained during 1 month and then decreased progressively until the beginning of September with some fluctuations of concentration, the elevated level remaining significantly higher than that of the controls ($P < 0.005$) from June to the beginning of September. From mid-September onwards the t-GTH level was not significantly different from the control.

When castration was performed in mid-September, t-GTH increased in only three out of six castrated males. High mortality reduces the validity of this experiment though the survivors' level of plasma t-GTH remained higher than in intact controls for at least 65 days.

However, castration in October and December induced a significant ($P < 0.01$) rise of t-GtH in plasma after 13 and 7 days respectively. In December, some animals were found with extremely high values of t-GTH (extremes 18 to 110 ng/ml of plasma). In addition to these results, Billard *et al.* (1976) described a slight response to castration in mid-May prior to the reproductive season when testicular development is feeble which contrasts with the response observed in some mammals (Davis and Meyer, 1973). This shows that in trout the hypothalamo-pituitary axis is under negative gonadal feedback control even outside the breeding season.

Steroids are probably among the gonadal factors which are responsible for this negative feedback at the hypothalamo-pituitary level, though little reliable information is available on the steroid levels in the blood during the teleost reproductive cycle. Other gonadal factors such as inhibin found in mammals (Setchell and Main, 1974) may also be involved in the negative feedback but since no information is available in fish, other experiments should be carried out before undertaking further discussion.

A better knowledge of peripheral and testicular levels of steroids and experiments including injection of steroids and testicular extracts and implantation of steroids and

testis tissue may help to evaluate the response of the hypothalamo-hypophyseal complex to castration.

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