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SOME FACTORS CONTRIBUTING TO THE DEVELOPMENT OF FUNGUS INFECTION IN FRESHWATER FISH

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Abstract: The intravenous administration of 1 mg of: cortisol, cortisone, estradiol or progesterone, or the infusion of 5 i.u./kg body weight/24h of thyroid stimulating hormone, had a facilitating effect upon fungus growth on the freshwater teleost *Catostomus commersonii commersonii* Lacépéde. The same dose (1 mg) of corticosterone or testosterone or adrenocorticotrophin (5 i.u./kg/24h) did not have this facilitating effect.

The possible role of steroids in the mechanism of action of temperature in promoting infections in fish is discussed.

INTRODUCTION

The majority of fungi involved in diseases of fish are members of the family *Saprolegniaceae*, the common "water moulds". They are characterized by a myceloid, eucarpic thallus bearing numerous reproductive cells. The most ubiquitous of the aquatic fungi, commonly attacking fish, is the eurytherm, euryhaline genus *Saprolegnia*.

Most authors agree that *Saprolegnia* is not in itself a specific disease but rather makes its appearance on individuals weakened by other maladies.[15] Stuart and Fuller[22] however, do not accept the view that the fungal infection is secondary. They state that *Saprolegnia* can act as a lethal primary parasite of healthy fish and that this genus alone has been isolated constantly from lesions of fish with ulcerative dermal necrosis (UDN).

Thus, the predisposing factors, contributing to development of fungus on freshwater fish are still controversial. One precondition appears to be the presence of bruises, wounds or abrased surfaces which provide the substratum for the growth of the fungus. But the importance of other predisposing factors such as stress[23] and water temperature cannot be ignored.

Since stress causes the release of stress hormones (e.g. cortisone) from the interrenal tissue[23] it seems possible that corticosteroids are involved in the etiology of fungus growth. Robertson *et al.*[17] showed that intraperitoneal implantation of cortisol pellets to immature rainbow trout resulted in skin infections and death. This appears to support the view that experimental administration of corticosteroids might have an effect similar to stress itself.

MATERIALS AND METHODS

Adult white suckers *(Catostomus commersonii commersonii* Lacépéde), weighing between 860 and 1150 g, were kept in large holding tanks using recycled, filtered, dechlorinated, aerated water. The temperature was held at 5 C during winter and 10 C during summer. The fish were not fed.

The operative procedures were similar to those used by Hunn *et al.*[10] and Mackay and Beatty.[12] The experimental fish were anaesthetized with 0.2 g/1 M.S. 222 (tricaine methanesulfonate, Sandoz) and subsequently catheterized with a Clay-Adams PE 200 polyethylene catheter introduced into the mesonephric duct and sutured into place with monofilament nylon. A PE 50 polyethylene tubing was introduced into the caudal vein for the purpose of injecting hormonal compounds. Following cannulation and catheterization each fish was placed in a specially built lucite box provided with

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constant temperature water. Both the catheter and the cannula were led out of the box through watertight fittings.

Some of the experimental fish were kept at 5 C (group E_1) while another group was kept at 13 C (E_2). They were injected once with 1 mg of one of the following pure, crystalline steroids: corticosterone, cortisone or cortisol (male and female fish), estradiol or progesterone (females only), or testosterone (males only). The particular crystalline steroid was dissolved in 0.03 ml purified ethanol and 1 ml of a mixture of: 2 parts Hickman's fish saline[8] and 1 part propylene-glycol, was added. This solution was injected through the caudal cannula. One ml of saline was used to wash the solution into the blood stream.

Since the average weight of the fish was 1010 g, the one mg steroid injected gave an average dose level of 0.99 mg/kg body weight (range: 0.87 - 1.16 mg/kg) and an estimated mean blood level (assuming blood volume approximately 5% of body weight) of 1980 μg% (range: 1730 - 2320 μg%).

Some of the experimental animals were treated with adrenocorticotrophin (ACTH) or thyroid stimulating hormone (TSH). ACTH or TSH were administered as continuous infusion (0.005 i.u./g body weight /24[h]) by means of a syringe-pump, for periods of 5 or 8 days.

There were two major groups of control fish: group A consisted of the intact fish kept in the holding tanks (group A_1 at 5 C and A_2 at 10 C); group B comprised fish submitted to the same operative procedures as the experimental animals, except that they were injected with the solvent mixture only, without added steroids. The group B fish were subdivided into smaller groups and kept at the following temperatures: 5 C (subgroup B_1), 10 C (B_2), 13 C (B_3) and 16 C (B_4).

RESULTS

During preparatory work it was noted that most of the fish kept at temperatures exceeding 10 C developed fungus growth. Based on this experience and assuming that initially the fungus invades only the very surface of the skin, where it is still accessible to topical treatment, each newly caught fish was dipped for 30 sec. into a trough containing a 1% solution of malachite green and 0.5 g/gal M.S. 222 (to keep the fish quiet) cooled to 5 C. This treatment was repeated once more, just prior to cannulation and catheterization.

Fungus development was absent on the fish kept intact in the holding tanks (Table 1) as well as on the control groups B_1, B_2 and B_3 even 30 days after cannulation and catheterization. Group B_4, however, presented fungus growth after a latency period of 10-15 days following operation. The experimental fish (E_1 and E_2) treated with estradiol, cortisol, cortisone or progesterone as well as those treated with TSH consistently developed fungus infection after a latency period of 7-10 days. Conversely, those injected with corticosterone or testosterone or infused with ACTH did not show any sign of fungus growth.

These results were the same for fish caught ripe in April and for those caught during the summer and winter months.

DISCUSSION

The mechanism of action of temperature in promoting infections in fish is still a matter of controversy. The majority of authors[1,14,23] reported a better immune response at higher than at lower water temperatures, and the rate of healing of UDN lesions was found to be faster at higher temperature.[16] On the other hand bacterial infections may be caused[3] and an epizooty accentuated[13] by increased water temperature. Cushing[5] reported that although the antibody-titer in freshwater fish increased faster at 28 C than at 15 C, the end-titer was higher at 15 C.

That the link between temperature and immunity control might be found in the adrenal cortical hormones was suggested by Bisset[4] who pointed out that the production of these hormones is inhibited at low temperatures. In agreement with this is the work of Fontaine and Hatey[9] who reported that ACTH restored to normal the interrenal of the

TABLE 1. Fungus growth on white suckers kept at various water temperatures, prophylactically treated with malachite green and injected (or infused) with various hormonal compounds.

Fish group	Sex	Water temp.	Operative procedure	Presence (+) or absence (—) of fungus growth							
A_1	M,F	5 C	None	—							
A_2	M,F	10 C	None	—							
				Injected with solvent mixture only							
B_1	M,F	5 C	cannulated and catheterized	—							
B_2	M,F	10 C	idem	—							
B_3	M,F	13 C	idem	—							
B_4	M,F	16 C	idem	+							
				Injected with						Infused with	
				estra-diol	cortico-sterone	cortisone	cortisol	testo-sterone	proge-sterone	TSH	ACTH
E_1	M	5 C	idem		—	+	+	—		+	—
E_1	F	5 C	idem	+	—	+	+		+	+	—
E_2	M	13 C	idem		—	+	+	—		+	—
E_2	F	13 C	idem	+	—	+	+		+	+	—

hypophysectomized eel at 16 C but not at 6 C. Robertson *et al.*[17] found that when the water temperature was raised from 13 to 17 C, death occurred much faster and with smaller doses in fish implanted with cortisol pellets.

In the present study temperatures exceeding 10 C appeared to make the suckers more susceptible to fungus infection than lower temperatures. This might have been the result of a disruption of the balance between host and bacteria causing infections predisposing to fungus development[2] or of the increased invasive activity of the fungus. Nevertheless, based on the findings mentioned above[4,17] and on the results of the present study, it is suggested that temperature acts mainly indirectly, through humoral factors, upon the immunological defense mechanisms of the fish. High temperatures will reduce the amount of transcortin-bound corticosteroids in fish plasma[7] and release free, biologically active steroids.[20] Thus temperature may be considered a permissive element which allows the activation of the immunodepressant interrenal corticosteroids.[23]

Although the glucocorticoids and particularly the 17-hydroxy-corticosteroids (17-OHCS) are traditionally known as restrainers of protein synthesis and stimulators of the catabolism of the amino acid building blocks of protein and thus of antibodies, nothing of the kind is known about estrogens or progesterone. On the contrary, estradiol is known to stimulate the synthesis of RNA and proteins in mammals. In the present study, however, estradiol administration was followed by fungus growth in the experimental fish. It seems possible that this effect was due to the triggering of free cortisol secretion, a supposition suggested by my own finding that estradiol injections caused excretion of free cortisol in the white sucker and by the report that humans treated with estrogens showed a marked increase of plasma 17-OHCS.[19]

The amounts of steroids injected in the present study were pharmacological rather than physiological, resulting in an initial blood level ten times higher than that found in spawned salmon plasma.[11] Although this high plasma level is maintained for a short time only, substantial amounts of the injected steroids or their metabolites may be detected in various organs of these fish even after 5 days from the time of injection.[18] The effects elicited by the presence of these high levels of steroids may be equated with those of stress[23] which, through a very similar mechanism, causes metabolic changes contributing to increased susceptibility to infection and significant reduction of immune response.[24]

ACTH did not show any effect upon fungus growth facilitation, in the present study. Whether this was due to the relatively low dosage used or to some other factor, as e.g. the possible formation of anti-ACTH antibodies during protracted treatment, is unknown. The role of ACTH in the various fish species needs much more study.

In conclusion it may be asserted that high plasma levels of 17-OHCS, estradiol, progesterone or TSH have a permissive, facilitating effect upon the occurrence of fungus infection in the white sucker fish. It is suggested that this effect is related to the impairment of antibody formation[9] and the suppression of reactive tissue inflammation.

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