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THE EFFECT OF *EDWARDSIELLA ICTALURI* INFECTION ON PLASMA CORTICOSTERONE LEVELS IN CHANNEL CATFISH *(ICTALURUS PUNCTATUS)*

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ABSTRACT

Channel catfish *(Ictalurus punctatus)* were innoculated with a new host specific bacterium, *Edwardsiella ictaluri*, to observe the influence of bacterial infection on plasma corticosterone levels at various temperatures. The fish were innoculated intraperitoneally. The infected fish were separated from the controls. Plasma corticosterone concentrations were determined by radioimmunoassay.

The plasma corticosterone concentrations in non-innoculated catfish were about 6.15 ng/ml and nearly 5.63 ng/ml in the infected fish. The lower level of the hormone in the infected catfish was not significantly different from the control level. High temperature was a stress factor which increased plasma corticosterone levels whereas *E. ictaluri* retarded the response of corticosterone secreting cells of the fish kidneys.

INTRODUCTION

Edwardsiella ictaluri is a bacterium which causes a deadly disease called "Enteric Septicemia of Catfish." This organism was identified and named by Hawke in 1979 when the disease erupted in the channel catfish farms of Georgia and Alabama. Hawke reported that the disease produced listlessness, spinning, external petechial hemorrhages of throat and mouth regions, hypertrophies of kidneys and spleen, hemorrhagic necrosis of liver and petechia of viscera, dorsal musculature and intestine. Death of the fish followed these signs within two weeks when the disease was waterborne or within 96 hours if the bacterium was innoculated into the peritoneal cavity.

The rapid advance of the pathological changes culminating in the death of the infected fish indicated total impairment or suppression of the host's immune and inflammatory mechanisms. These defense systems are often suppressed in animals by high concentrations of corticosteroids. According to Hans Selye (1976) the adrenal cortex would secrete these hormones excessively if the animals are stressed. The inhibitions of immune responses and inflammatory responses in channel catfish could be due to this reason.

Regardless of the structural and anatomical peculiarities of the ductless glands in fish, the fish endocrine system is functionally similar to that of the tetrapods. There are no distinct adrenal glands in fish. Instead, the corticosteroids are secreted by the acidophilic cells of the interrenal tissue in these animals. One or more layers of the cells form a sheath around the posterior cardinal veins and their branches which ramify within the cranial regions of the head kidney (Hoar, 1957). Gorbman and Bern (1962) confirmed that these cells secreted cortisone, cortisol, corticosterone, and dehydrocorticosterone. Selye (1949) observed that the fish pituitary and interrenal tissue responded to environmental stress by increased production of the glucocorticoids after the same manner as the responses to obnoxious stimuli by the hypophysis and the adrenal glands of higher animals. According to Selye (1976) environmental factors such as salinity changes, temperature extremes, captivity, crowding, muscular fatigue, hypoxia, hemorrhage and trauma can influence the glucocorticoid secreting cells to increase the production of the hormones. Significant increases in the plasma levels of glucocorticoids due to stress were observed in fish by Rasquin and Rosenbloom (1954), Hoar (1957), and by Ball and Slicher (1962).

The effect of temperature on plasma corticosteroid concentrations in channel catfish was studied by Davis et al. (1979). They reported 10 ng/ml as the baseline value of total corticosteroids in catfish held in an aquarium at room temperature. In water at or below 10° C the level of plasma corticoids rose to a range of 18.7 to 40.7 ng/ml. The concentrations of corticoids in the plasmas of channel catfish decreased to a value between 4.1 to 6.1 ng/ml when the fish were transferred to water temperatures of 15 to 20° C.

In a study on *Oncorhynchus kisutch*, an increase in serum cortisol and a decrease in interrenal ascorbic acid were indicators of non-specific stress in temperature shock at 10 ± 7° C (Wedemeyer, 1969). Low temperature slows down metabolic and osmoregulation rates, central nervous system responses, swimming speed, and feeding (Umminger and Gist, 1973). Fish acclimated to a low temperature have higher total tissue oxygen consumption rate. The liver, skeletal muscle and stomach have a higher oxygen consumption rate than the brain under these conditions (Rieck et al., 1960). Compared to channel catfish maintained at 20° C under the same conditions, those maintained at 15° C and confined in a net had a longer lag phase, lower active secretion phase, and a lower maintenance phase in plasma corticosterone levels (Davis et al., 1979). Goldfish acclimated to 10° C demonstrated more symptoms of stress due to handling and sham injection than did fish acclimated to 32° C. High temperature studies revealed that females are usually more heat tolerant than males. There is no difference in heat tolerance between a spawning female and a non-spawning female, but there was diurnal rhythm in heat resistance (Johnson, 1976).

In fish the thermoregulatory center is located in the rostral brainstem. These animals change their body temperatures by heat exchange with the environment. They move from an area of higher than optimum water temperature to cool regions in an apparent effort to maintain a fairly constant body temperature (Crawshaw and Hammel, 1974) which is the "preferred temperature" in which the species functions physiologically and becomes evolutionarily adapted (Umminger and Gist, 1973; Brett, 1971). Rapid temperature changes can bee detrimental to poikilotherms including fish. The temperature which is fatal to 50% of a population is defined as the lethal temperature. The fish with the lowest thermal tolerance is the *Oncorhynchus* and the most eurythermal is the Ameriuridae (Brett, 1956).

Channel catfish *(I. punctatus)* have a narrow range of temperature tolerance. Their upper lethal limit is 33° C if acclimated gradually (Love, 1980). In *I. lacustris,* the upper lethal limit is 33.5° C and the lower lethal limit is 0° C (Brett, 1956). When the temperature reaches 23.0° C from mid-May to mid-June in Arkansas, channel catfish spawn (Brady, 1981). Channel catfish optimum digestion temperature is 26.6 to 29.4° C and they are most active at this heat level (Shrable, Tiemeier, and Deyoe, 1969).

The common bacterial diseases of channel catfish are *Pseudomonas flourescens, Aeromonas hydrophila, Flexibacter (Chondrococcus) columnaris,* and *E. tarda* (Hawke, 1979). *Aeromonas hydrophila* and *F. columnaris* make up 82.8% of all bacterial cases in fish. Bacterial diseases appear between April and September while viral diseases may

appear between June and September (Plumb, 1975). The bacterial onslaught occurs in the southern United States concurrently with the optimum temperature for *E. ictaluri* (Hawke, 1979). Environmental stress such as high temperature and poor water quality caused by overcrowding, over feeding, over fertilization, chemicals, algae die-offs, and seasonal water upheavals predispose fish to bacterial infections (Walters and Plumb, 1980).

The endotherms are known to develop fever in response to bacterial infection. The ectotherms require a higher than normal environmental temperature when they are infected by microorganisms. The desert iguana *(Dipsosaurus dorsalis)*, goldfish *(Carassius auratus)*, largemouth black bass *(Micropterus salmoides)*, and bluegill sunfish *(Lepomis macrochirus)* when injected with killed *Aeromonas hydrophila* moved to an area with a higher temperature. *D. dorsalis* regulated its body temperature by walking back and forth between a cool and a warm chamber (Vaughn et al., 1974). This movement is a behavioral adaptation to absorbe heat from the environment and to increase the body temperature until it was in equilibrium with the hypothalamic thermal set point which was raised by pyrogens (Covert and Reynolds, 1977). Endotoxin secreted by the bacteria triggers the release of endogenous pyrogens and prostaglandins which influence the hypothalamus to demand an elevated body temperature (Vaughn et al., 1974; Reynolds et al., 1976; Kluger, 1978). It has been postulated that at the high temperature, the immunological system is accelerated to synthesize immunoglobulins and enhance phagocytosis (Covert and Reynolds, 1977). Goldfish respond to bacteria by the production of precipitating agglutinins and neutralizing antibodies in the blood (Chavin, 1973). Thus, fever seems to have a protective effect on animals because fish that are allowed to thermoregulate behaviorally have no mortality when compared to those with no choice of temperature (Covert and Reynolds, 1977).

The purpose of this study was to determine the plasma corticosterone concentrations of channel catfish *(Ictalurus punctatus)* which are innoculated with *Edwardsiella ictaluri* and held in various water temperatures, and to observe if stress was a cause for the immune deficiency of these animals.

MATERIALS AND METHODS

Yearling channel catfish *(Ictalurus punctatus)* were obtained from Kueter's Lake, T 16 N, R 6 E, S 8 and 90° 27 ' W by 36° 2 ' S, Paragould, Arkansas. They were held in indoor fish tanks at a density of 15 fish per 180 liters of water. The water was aerated and filtered continuously with electric air pumps and bottom filters. Constant temperature of 21° C, 23° C, 25° C, 27° C and 29° C were maintained in separate aquaria by means of thermostatic heaters. For each of these temperatures there were two groups of fish namely the controls (n = 15) and the infected (n = 15). Prior to the start of the experiment the fish were acclimated to the assigned temperature. These animals were not fed during the experiment.

On day one at the beginning of the experiment the experimental fish were anesthetized by immersion in an 80 ppm solution of MS-222 and by means of a tuberculine syringe fitted with 25 × 5/8 needle they were innoculated intraperitoenally with *Edwardsiella ictaluri* supplied by Stoneville Research Laboratories, Stoneville, Mississippi. The dose was 0.05 ml containing approximately 1.3×10^7 organisms. The fish were allowed to recover from anesthesia before returning them to the aquaria. The control fish were anesthetized, allowed to recover and were returned to aquaria.

At 7:00 p.m. on day three and at the same hour of the following days the fish were serially sacrificed to collect blood and tissue samples. For this purpose, a fish was gently lifted from the water with a hand net and was stunned by a hard knock on the head. Quickly blood was drawn from the fish by cardiac puncture into a heparinized syringe. Minimally about 1 milliliter of blood was obtained by this method. The blood was transferred to heparinized vacutainer tubes and was centrifuged at 3000 rpm in an International Clinical Centrifuge. The plasma was pipetted into labelled vials and was stored at − 10° C until assayed. The fish was grossly examined and the observed lesions were described. Also, samples of liver, spleen, and kidney were collected for bacterial count.

The plasma was thawed and was mixed with 15 ml of methylene chloride by shaking the tubes for fifteen minutes. The mixture was filtered through #2 Whatman Filter Paper and the filtrate was collected and dried under a current of dry air. The residue was redissolved in 1 milliliter of methanol and the amount of corticosteroid in this solution was determined by radioimmunoassay using a corticosterone specific antiserum prepared by the Endocrine Sciences, Tarzana, California.

The data collected in this study were analyzed by Student t-test and by two-way analysis of covariance to determine the significance of differences (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The body weights of the control catfish and the experimental catfish were similar. There was no marked variation of the body length between the controls and the innoculated. Consequently the body weight to body length ratios of the innoculated fish were not markedly different from similar ratios of the controls (Table 1).

The infected fish had petechia of the throat and mouth skin areas. Similar lesions were observed on the internal surface of the body wall and on the external surfaces of stomach and intestine. The control fish were not affected by similar lesions. Bacterial counts were available from the livers, spleens, and kidneys of the infected fish only (Table 1).

The plasma corticosterone levels of the fish in this study are presented in Table 2. The plasmas of the controls and of the experimental fish from 21° C water, contained similar amounts of corticosterone. In the fish from 23° C environment, the plasma concentrations of corticosterone were not significantly different when these values of the innoculated fish were compared to those of the controls. At 25° C water temperature the infected fish had no markedly varied amounts of circulating compound B in comparison to the quantity of the same substance in the control fish. The 27° C controls and innoculated catfish showed no significant difference of plasma corticosterone concentrations. The catfish maintained at 29° C had high concentrations of plasma corticosterone; but these levels in the innoculated fish were not statistically different from the control levels at that temperature.

There was a significant difference which was observed when the plasma concentrations of corticosterone in the fish maintained at 21° C was compared to the similar values of the fish living in 29° C water, whether the fish were infected by *E. ictaluri* or not ($P = 0.01$).

According to the data obtained from this study the channel catfish developed "Enteric Septicemia of Catfish" when *E. ictaluri* was introduced into them under experimental conditions. The infection was fatal to these animals as reported by Hawke (1976). However, there was no increase or decrease of circulating corticosterone due to *E. ictaluri* infection in channel catfish. There was a significant increase of Compound B in the fish maintained at 29° C, when compared to the amounts of this hormone in the plasmas of fish living in 21° C environment.

The plasmas for the determination of corticosterone concentration were collected at 7:00 p.m., the hour at which the hormone reached the peak value of diurnal rhythm as observed by Boehlke, Tiemeier and Eleftheriou (1966). The amounts of corticosterone in the plasma samples were determined by radioimmunoassay because of its specificity, reproducibility and sensitivity. Using this method the fish plasmas contained about 6.15 ng/ml of plasma. This value is similar to the plasma level of corticosteroids determined by Davis et al. (1979) in fish of the same species and age maintained in water between 15-20° C. Davis et al. (1979) determined the corticosterone concentration in fish plasma by the "competitive protein binding method" which is closely related to radioimmunoassay.

The control catfish did not possess any bacteria in the kidneys, liver or spleen. In the innoculated catfish these organs were infected. Therefore, the functional efficiency of these tissues could be impaired.

The amount of corticosterone in catfish plasma as reported by Boehlke et al. (1966) was very high when compared to the values obtained by Davis et al. (1979). The fish in the experiment conducted by Boehlke et al. were older and heavier ranging from 350 to 550 g. Also, the plasma corticosterone in them was assayed by a fluorometric method which

Table 1. Body lengths, body weights, and density of *Edwardsiella ictaluri* in different tissues of channel catfish *(Ictalurus punctatus)* maintained at various temperatures.

Temperature centigrade	Group	Number	Body Length centimeter	Body Weight gram	Body Weight / Body Length	Average $\log_{10}$ of *Edwardsiella ictaluri* per gram of tissue		
						Kidney	Liver	Spleen
21	I Control	7	16.64 ± 4.74	68.09 ! 8.71	4.09	----	----	----
	II Innoc.	10	19.15 ± 0.52	90.44 ± 6.37	4.72	7.77	7.66	8.13
23	III Control	3	17.13 ± 1.43	69.38 ±21.18	4.05	----	----	----
	IV Innoc.	12	20.29 ± 0.32	111.71 ± 5.87	5.51	8.05	8.04	8.58
25	V Control	15	19.09 ± 0.46	93.17 ± 6.13	4.88	----	----	----
	VI Innoc.	12	21.31 ± 0.36	120.71 ± 5.31	5.66	7.23	8.85	9.56
27	VII Control	13	15.99 ± 0.55	55.37 ± 5.54	3.46	----	----	----
	VIII Innoc.	9	16.56 1 0.81	66.58 ±11.49	4.02	9.40	8.27	10.08
29	IX Control	14	21.81 ± 0.37	127.44 ± 5.98	5.98	----	----	----
	X Innoc.	13	21.20 ± 0.38	118.95 ± 4.91	5.61	8.79	8.84	9.44

Table 2. Effect of temperature on plasma corticosterone concentrations of channel catfish infected by *Edwardsiella ictaluri*

Temperature ° C	Number of fish (control/innoc.)	Corticosterone Concentration ng/ml of plasma Mean Standard Deviation	
		Control	Innoculated with *Edwardsiella ictaluri*
21	7/7	4.54 ± 1.21[a]	3.52 ± 1.04[c]
23	3/3	6.45 ± 1.09	4.89 ± 1.18
25	12/12	4.22 ± 1.33	5.73 ± 1.57
27	9/9	7.63 ± 2.39	5.89 ± 3.36
29	12/12	7.84 ± 2.93[b]	6.73 ± 1.70[d]

a vs b P = 0.01

c vs d P = 0.01

is not as specific as the method of competitive protein binding or radioimmunoassay.

There was no significant effect of bacterial infection on plasma corticosterone concentration in channel catfish. However, the plasma levels of compound B in the infected catfish were consistently lower than the control values for fish maintained at 21, 23, 25, 27, and 29° C. The decreased corticosteroid secretion by infected catfish can be related to the pathological changes observed in the kidneys of these animals. Whether or not the interrenal tissue in these animals had lost their function was not established by challenging the endocrine gland with exogenous ACTH.

The plasma corticosterone concentration can vary in animals from time to time according to the changes in the rate of secretion, metabolic utilization, protein-binding, and renal excretion. The change in the rate of secretion of adrenocorticoids in animals is regulated by the hypothalamo-hypophyseal adrenal axis by a negative feedback mechanism which can be stimulated by emotional, environmental, mechanical, or chemical factors (Zarrow, Yochim, and McCarthy, 1964). Generally, a significant increase in the secretion of these hormones occur when animals are stressed by one or more of these factors (Selye, 1949). According to this theory, the animals in this study did not show a significant increase in the secretory rate of plasma corticosterone due to the infection. The increase in temperature of the fish tanks did cause increased plasma corticosterone concentrations in these fish. Previously it was reported that a decrease in environmental temperature caused a rise in the level of plasma corticosterone in channel catfish (Davis et al., 1979). Therefore, cold and hot environments seem to be stressful to catfish.

CONCLUSION

The suppressions of immuno response and inflammatory reactions in channel catfish infected by *Edwardsiella ictaluri* are not the results of excessive amount of circulating corticosterone. The environmental temperatures, except 29° C, were not stressful to the fish in this experiment. Hence, the failure of the defense mechanisms during bacterial infection in channel catfish is not due to the action of stress on the hypothalamic hypophyseal adrenal axis.

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