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Dose Dependent Alcohol Effects on the Aggressive Behavior of the Convict Cichlid (*Cichlasoma nigrofasciatum*)[1]

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Previous research has indicated that the fish is a useful model system for research on the effects of alcohol on behavior. The present investigation examined the effect of known blood levels of alcohol, as ascertained from uptake data reported here, on conspecific aggression in the convict cichlid. In different groups, known doses of alcohol (0.2 and 0.5%) were added to a tank containing fish. At various intervals up to 6 hr, fish were sacrificed and the alcohol concentration of their blood compared with the tank ethanol concentration. The results indicate that 6 hr is required for the blood concentration to approach equilibrium with the tank at an average of 65% of the tank concentration. To investigate the effects of various alcohol doses (0.07, 0.18, or 0.33%) on the aggressive behavior of a single male convict cichlid, an intruding male was introduced into the resident fish's tank in a glass tube. At the highest ethanol dose almost half of the fish did not attack the intruder. Those fish which did attack were significantly less aggressive than fish in any of the other groups. The intermediate dose (0.18%) produced significantly more aggresssion than was observed in controls or at higher or lower alcohol doses. These results indicate that ethanol enhances aggression at moderate doses and reduces it at high levels.

Studies of intraspecific aggression in nonhuman species can provide much important information regarding the biological basis of aggressive behavior. A "model system" to be used in such investigations must be appropriate both from a behavioral point of view and a physiological one

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when the dependent variables are physiological manipulations. Fish of a number of genera display much aggression in defense of territory, and such aggression can be reliably elicited in a laboratory situation. Combined with the appropriateness of the fish as a behavioral model is the fact that the fish is an ideal subject for studies of alcohol effects on behavior (Ryback, 1970). Ryback *et al.* (1969) studied blood alcohol levels as a function of time of exposure to ethanol in the aquarium water and determined the rate and steady state level of uptake of the alcohol in the goldfish (*Carassius auratus*). They demonstrated that the steady state level of alcohol was a constant percentage of the aquarium concentration.

Implicitly assuming that uptake would be the same in all species of fish, Raynes and Ryback, in a series of experiments (Raynes *et al.*, 1968; Raynes and Ryback, 1970), studied the interaction of ethanol and aggression in the Siamese fighting fish (*Betta splendens*). The results of these studies indicated that 0.29% (0.29 g/100 ml) ethanol in the water increased the amount of aggression that the fish directed at their own mirror image and that fish under this alcohol dosage would withstand more punishing electric shock than controls before suppressing aggressive responses to a conspecific. They further showed that very high doses of ethanol (0.65%) in the water decreased aggressivity. But since the rate of uptake and terminal level of alcohol was not determined in this species, the actual blood level of alcohol in these experiments is uncertain.

In addition, using a mirror image to release intraspecific aggression as was done in these studies has been shown to be an unnatural and abnormal stimulus. A complete descriptive analysis of the aggressive display of *Betta splendens* by Simpson (1968) revealed that normally one *Betta* faces a second that stands at right angles so that rarely are the two fish parallel to each other or facing each other as in a mirror image.

The aggressive behavior of the species used in the present investigation is well-known, has been studied in our laboratory (e.g., Peeke *et al.*, 1971; Gallagher *et al.*, 1972) and can be elicited more reliably by natural stimuli than by mirror images, i.e., a live conspecific intruding in the subject fish's territory. Also, by recording several measures of aggressive behavior, it is possible to observe changes in multiple response topographies as well as diminutions and increases in any one response.

The present investigation was designed to clarify and extend previous work demonstrating a relationship between ethanol and aggression in a biologic model system: the fish.

EXPERIMENT I ALCOHOL UPTAKE AND BLOOD LEVEL

The first experiment was designed to ascertain the rate of uptake and steady state level of blood alcohol in the Convict Cichlid over a range of water-alcohol concentrations. The concentrations chosen for study, 0.2 and 0.5% overlapped those used by Ryback *et al.* (1969) and were used in the hopes of replicating their findings that blood to water alcohol ratios (percentage) would be similar for all dosages.

Methods

The desired weight of alcohol (95% USP) was added to give the required concentration in an 84-liter aquarium containing 20-30 adult male convict cichlids. Initially, and at intervals thereafter (30, 60, 120, 240, and 360 min), samples of the tank water and blood from the fish were assayed for alcohol content. The fish were rendered unconscious by a sharp blow to the skull, decapitated, and blood taken up from the heart cavity by capillary action into heparin coated "Crito-Tubes" (capillary glass tubes designed for determination of red cell hematocrits). The tubes were weighed before and after filling; they were washed into 0.3 ml deionized water, and this diluted sample used for the assay. Several "kit" procedures were used as described by the manufacturer; they produced equivalent results. They are based on the dye coupled-NAD-alcohol dehydrogenase reaction, as originally described by Bonnichsen and Theorell (1951). Sensitivity was adequate to reliably measure blood content of 0.03% ethanol in 15 mg of blood.

Results

As shown in Fig. 1, the ethanol content of the fish blood approached the tank level only after 5-6 hr. Sufficient numbers of fish (6-15) were examined at each time, so that the blood level at any time can be described as

$$L = L_f(1 - e^{-kt})$$

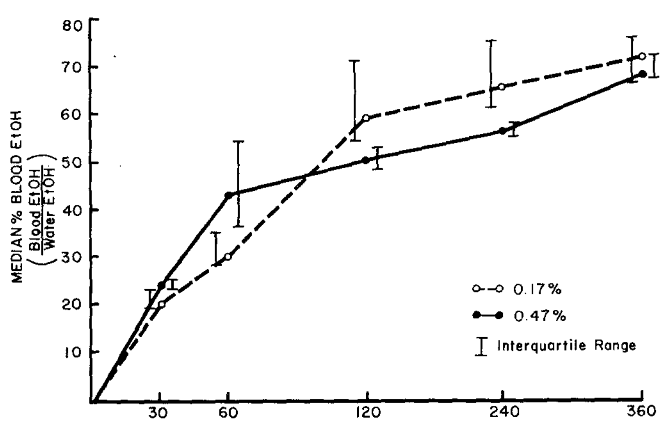

Fig. 1. Blood ethanol level in fish immersed in 0.17 or 0.47% ethanol solutions for 0.5, 1, 2, 4, or 6 hr as a proportion of aquarium ethanol concentration.

with standard deviation of 12.2%, where L is the blood ethanol level of the fish, L_f is the final blood level (see later), and k is 0.01/min (i.e., a half-time for the absorption of about 70 min). The final blood concentration (L_f) was not equal to the tank concentration, but averaged 65% of that concentration for both the low and high dose. This observation suggests that there is a mechanism for "pumping" alcohol into the tank from the fish. The simplest mechanism for describing these changes in alcohol concentration then is:

$$\text{Alcohol (aquarium)} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{Alcohol (fish)}$$

$$\text{Alcohol (total)} = \text{Alcohol (aquarium)} + \text{Alcohol (fish)}$$

From these data, we estimate that $k_1 = 0.0065$ and that $k_2 = 0.0035$. From similar data presented by Ryback *et al.* (1969), $k_1 = 0.0085$ and $k_2 = 0.0015$. It is clear from these data that generalizing uptake and blood level data from one species of fish to another is hazardous. It also appears that 6-8 hr may be required for blood ethanol to approach equilibrium with tank concentrations.

EXPERIMENT II ALCOHOL EFFECTS ON AGGRESSIVE BEHAVIOR

From the results of Expt. 1, it is clear that over a broad range of alcohol concentrations uptake rate and terminal percentage in blood are the same. In addition, less variability was apparent at 6 hr although the mean level is slightly higher than that seen 2 hr earlier. Armed with these results the question of dose-response functions between blood-alcohol levels and aggression could be addressed.

Adult male convict cichlids were individually placed into 57-liter glass aquaria with coarse gravel covering the floor. A ceramic flower pot lying on its side in the center of the aquaria provided refuge for the fish. Air-driven filters provided filtration and circulation, but were disconnected when alcohol was introduced into the tanks. Tanks were maintained at 26° C (± 2° C) and fish were fed dry food daily. After a 4-day acclimatization period, alcohol was introduced into the fish's aquarium in one of three planned experimental dosages, 0.075, 0.20, or 0.35%; when measured by methods described before the observed water alcohol levels were 0.07, 0.18, and 0.33%. This discrepancy may be due to absorbtion by the flower pot and gravel and minor variations in aquaria water levels before introduction of the ethanol. Individual variations at dosage level were minor.

Six hours after introduction of the appropriate amount of ethanol, the resident fish was presented with a live, male conspecific which was confined to a clear glass tube (100 mm diam) filled with water at a spot 200 mm from the entrance to the resident fish's flower pot entrance. Two experimenters were situated in front of the aquaria, each with a hand console containing buttons which, when pushed, registered frequency and duration of various responses. One experimenter recorded responses of the resident fish while the other observed the behavior of the intruder. The responses recorded were: (1) frequency of displays, where a display was counted each time the fish extended gill covers and branchiostegal membranes; (2) duration of each display; and (3) frequency of bites at the glass tube containing the intruder.

The latency of the resident (subject) male fish to exit from his refuge was measured for each subject fish starting after the *E* had positioned the stimulus tube. A fish was permitted 8 min to exit from his refuge. If the fish attacked the intruder, the observation period was started and lasted for 8 min.

Results

Whether or not a fish engaged in aggressive behavior at all was taken as a measure of timidity. It was found that while latency to exit from the refuge did not differ among groups, the proportion of those remaining in their refuge or exiting and making no response did. The percentage of each group which attacked the intruder is shown in Fig. 2. It was found that all of the subjects

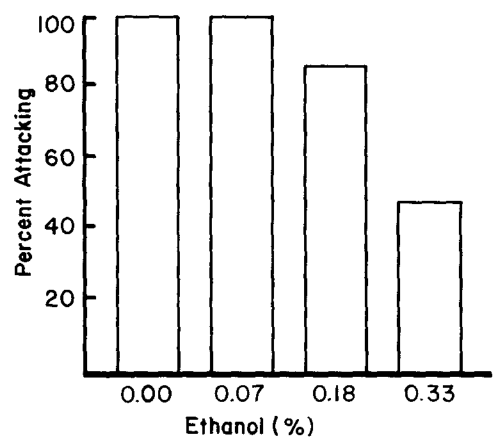

Fig. 2. Percentage of groups emerging from shelter and attacking intruding conspecific, as a function of tank ethanol concentration (0, 0.068, 0.184, or 0.33%).

in both the nonalcohol control group (n=12) and the low dose (0.07%) group (n =13) attacked. While 14 of the 16 medium dose (0.18%) fish emerged and entered into combat (85%), only 8 of 17 fish in the high dose group (0.33%) would attack. This indicates a progressive increase in "timidity" with increased alcohol level. That this effect was not due to motor impairment is supported by observations that even at the highest dose fish appeared perfectly able to swim normally and had no obvious signs of locomotor difficulty. It would appear from these data that the willingness to approach an intruding rival decreases with increased blood alcohol levels.

Analyses of the three measures of aggressive behavior were performed only on fish which emerged and attacked; hence, all subjects contributing to the tests and graphs (Fig. 3) showed some aggressive behavior toward the intruding rival.

With all of the responses measured, Bites, Display Frequency, and Display Duration, a similar pattern of results emerged, indicating that the relationship among the responses measures of aggressive behavior did not differ as a function of alcohol blood level. The over-all level of aggression, however, shows marked differences as a function of blood alcohol level as is indicated in Fig. 3.

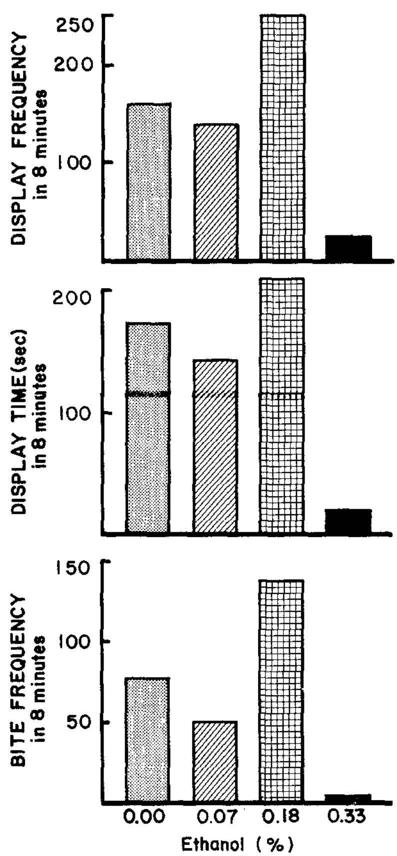

Fig. 3. Median frequency and duration of display and frequency of bites during 8-min observation periods as a function of tank ethanol concentration (0, 0.068, 0.184, or 0.33%).

There were no significant differences between the nonalcohol control group and the low dose alcohol group on any of the recorded behaviors, although with all three measures the median level of response was slightly less in the latter group. The medium alcohol dose group showed a higher level of aggressive responsiveness than either the control or low alcohol groups. (Control vs medium dose:[2] Display Frequency, $P < .01$; Display Time, $P < .05$; Bites, $.05 > P < .10$.) The highest dose of alcohol resulted in less aggressive behavior than was seen in any of the other three groups on all measures. Comparison between all other groups with the Display Frequency and Bites measures yielded significance in all cases ($P < .002$). With the Display Time measure, the high dose group was less aggressive than the control group ($P < .02$), the low dose group ($P < .02$) and the medium group ($P < .002$).

The pattern of results was quite similar across measures; thus, the dose-response relationship of ethanol and aggression with the cichlid fish appears to approach an inverted U. Low doses produced little effect, moderate ones increased aggression, and the highest inhibited it. Again, it should be emphasized that although the highest dose of alcohol reduced the level of aggression, there was no evidence that this dosage produced its effect through impairment of movement or locomotion.

DISCUSSION

The results of these experiments confirm and extend those of Ryback and his colleagues. In the present study it was shown that the rate of uptake and terminal level of blood alcohol is specific to the species under study, and consequently it appears hazardous to base assumptions or presumed blood alcohol levels in one species on uptake data from another. It was shown that the rate of uptake, expressed as a percentage of the tank water concentration, proceeds at the same rate regardless of the ethanol concentration, at least up to 0.3 g%.

The finding that as alcohol dose increased beyond the facilitating level fish were less likely to be aggressive parallels the results of MacDonnell and Ehmer (1969) who found that, with increased doses of intravenously administered alcohol (0.037-.015 g%), the latencies of attacks of cats on rats elicited by electrical stimulation of the hypothalamus were greatly increased and the attacks were less effectively carried out, i.e., incomplete attacks with missing components. Our finding that as alcohol level was increased, fish became more aggressive up to a point, and then less aggressive, is also similar to their observation that moderate levels of alcohol (0.025 g%) produced increases in

[2]Kruskal Wallis analyses of variance were performed across all groups for all measures and yielded values beyond the .01 levels in all cases. Comparisons between pairs of groups were made utilizing two-tailed Mann-Whitney U Tests.

the force of biting in response to stimulation,[3] and also supports the observation of enhanced aggression at doses of 0.285 g% reported by Ryback and his colleagues.

The observed effects in fish are analogous to the alcohol-aggression interactions in humans as noted in a study of urine-alcohol concentration in persons arrested during or immediately after the commission of a felony (Shupe, 1954). In this study of 882 persons, 75% of the people arrested for shooting and other assaults had alcohol levels between 0.10% and 0.39% while less than 20% had no alcohol and only 2% had concentrations over 0.4%. Questions regarding social effects of alcohol might best be reviewed in terms of societal overlays on the biological base, which when better understood, may provide the basis for a comprehensive theory of alcohol effects on human aggressivity. Any theory that ignores such bases will likely be incomplete as well as misleading.

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[3]The increases in force of biting are also quite consistent with the results of studies of the effects of alcohol on inhibition of the masseteric reflex in acute cats (Sauerland *et al.*, 1967). As blood alcohol levels increased from 0 to 0.67 g%, orbital-cortically induced inhibition of the masseteric reflex became progressively less. The masseteric reflex itself, in the absence of orbital gyral stimulation, decreased in amplitude with increases in alcohol dosage, suggesting that the alcohol was more effective in reducing inhibitory influences than in simply decreasing motor responses alone.