Effect of Tranquilizing Drugs on Fighting Response of Siamese Fighting Fish

With the development of psychotherapeutic agents, the need for a simple, inexpensive method for their evaluation at a behavioral level has become extremely acute. Some measure of success has been achieved by determining their effectiveness in suppressing a conditioned emotional response of fear or anxiety in rats and monkeys (*1*). In view of the fact that tranquilizing drugs, such as reserpine and chlorpromazine, tend to diminish aggressiveness in untamed animals, and, particularly, since they are presumed to act at subcortical levels, it seemed worthwhile to explore the effects of such agents on the inborn pugnaciousness of the Siamese fighting fish, *Betta splendens* (*2*). Previously, the effect of lysergic acid derivatives on these fish had been studied in detail (*3*).

The fish normally respond to one another by exhibiting a characteristic fighting stance, which involves the expansion of the pectoral fins and branchial membranes, and by viciously attacking one another, often to the point where one is killed (*4*).

Sexually mature male fish, all exhibiting the fighting response, were used in the present study. A total of 48 fish were used, and only in instances where the animals showed complete recovery after 10 days were they used again. In the case of each drug studied, only fresh animals were used (*5*).

The results represent the independent conclusions of all the observers, and only those results for which agreement was unanimous are reported. The experimental aquarium (rectangular) was made of an opaque plastic and divided into two chambers separated by a watertight transparent Lucite panel and a removable opaque panel. After the experimental fish had been sufficiently exposed to the drug, which was dissolved in one chamber, the sliding panel was removed, and the fighting response was observed. Whenever the response appeared to be negative, the experimental fish was placed directly in the control chamber for further observation. The results are shown in Table 1.

In addition to the tranquilizing agents, a number of pharmacologically related substances were tested, such as narcotics, sedatives, antihistaminics, and analgesics. On the basis of the fighting and other behavorial responses, the drugs seem to fall into four distinct groups: the reserpine-Meprobamate, the chlorpromazine, the antihistaminic, and the barbiturate classes.

Reserpine and Meprobamate were studied in doses of 1, 2, 5, and 10 μg/ml. The fish refused to fight, exhibited backward swimming movements, and showed no attacking response. However, they remained active, swam about, and had normal appetites. Complete recovery occurred in 48 to 96 hours. After being exposed for a few minutes to a dose of 2 μg of chlorpromazine per milliliter, the fish became sedated and assumed a "Cartesian diver" position (*3*). They were, however, reactive to stimuli and remained inactive until they were attacked by other fish, whereupon they swam away. Chlorpromazine sulfoxide, the detoxification product of chlorpromazine, produced no behavioral changes in concentrations up to 50 times greater than those of chlorpromazine.

The fish that were exposed to the antihistaminic drugs not only failed to show the fighting response, but tried to escape from and to avoid the attacking fish. They remained quiet until they were exposed to a fighting fish, from which they escaped with such violence that they frequently almost jumped out of the tank. This behavior was altogether different from the behavior of the tranquilized fish, which retreated rather slowly (and deliberately) from an attacking fish. The normal response to a female fish, which is characterized by graceful swimming movements and is accompanied by the fighting stance, was absent in fish exposed to the antihistaminics. When two fish that had been treated with the antihistaminics were placed together, they equally avoided one another, and neither exhibited the normal fighting stance. The action of the antihistaminics lasted a very long time, and even after a week in fresh water, the fish exhibited a similar response. Another peculiarity was that they became pale upon exposure to a control fish exhibiting the fighting response. When the fish were again isolated, their color would return. This effect could be seen 4 days after a single 1-hour exposure to the drug.

The barbiturates produced a definite sedative effect. After 2 hours in sodium phenobarbital, the fish rested on the bottom, but showed the fighting response when goaded. With thiopental, the fish were actually narcotized; but they could be readily aroused and would orient themselves temporarily toward the control fish as if to fight, and then relapse into a depressed state.

In sodium salycilate, the fish behaved normally, and perhaps slightly hyperexcitably. Morphine, in concentrations up to 40 μg/ml, had a somewhat similar effect, and, if anything, increased ag-

Table 1. Effect of various drugs on Betta splendens.

Drug and class	No. of fish	Concn. (μg/ml)	Onset of action (min)	General action	Fighting response	Type of behavior when confronted with normal fighting fish
Reserpine (tranquilizer)	8	10	120	Very slight depression	No	Retreated, usually backward; refused to fight
Meprobamate (tranquilizer)	5	10	120	Very slight depression	No	Retreated, usually backward; refused to fight
Chlorpromazine (tranquilizer)	8	2	10–60	Strong depression	No	Quiescent, but retreated when attacked; refused to fight
Chlorpromazine sulfoxide (detoxification product of chlorpromazine)	3	50		No action	Yes	Showed fighting response
Phenergan (antihistaminic)	2	20	2	Depression	No	Retreated very rapidly
Pyribenzamine (antihistaminic)	3	20	2	Slight depression	No	Retreated rapidly
Benadryl (antihistaminic)	3	5	30	Depression	No	Retreated very rapidly
Atarax (antihistaminic and sedative)	3	20	45	Slight depression	No	Retreated very rapidly
Sodium phenobarbital (hypnotic)	2	30	90	Depression	Yes	Will fight; quiescent
Sodium thiopental (hypnotic)	4	20	35	Strong depression	Yes	Will fight; quiescent
Morphine sulfate (sedative and analgesic)	2	40		Slight excitation	Yes	Will fight; very aggressive
Sodium salicylate (analgesic)	3	400		Slight excitation	Yes	Will fight; very aggressive

gressiveness. In all the experiments, recovery was complete, and there were no mortalities.

Although preliminary observations would indicate that *B. splendens* responds differently to a diverse number of pharmacological agents, the tranquilizing agents do seem to induce a characteristic response. All such agents definitely suppressed the quality of pugnaciousness without necessarily impairing sensitivity and motor activity. It is felt that this preparation can be used in the partial evaluation of the tranquilizers, as well as related neurotropic agents.

E. J. WALASZEK
L. G. ABOOD
Department of Psychiatry and
Department of Biochemistry,
University of Illinois
College of Medicine, Chicago

References and Notes

1. J. V. Brady, *Science* **123**, 1033 (1956).
2. This research was supported jointly by a contract between the U.S. Office of Naval Research and the University of Illinois and by a grant from the Illinois Department of Public Welfare.
3. H. A. Abramson and L. T. Evans, *Science* **120**, 990 (1954); L. T. Evans *et al.*, *Science* **123**, 26 (1956).
4. W. T. Innes, *Exotic Aquarium Fishes* (Innes, Philadelphia, Pa., 1955).
5. The observations were made independently by us and by two psychologists, Garth J. Thomas and Leon S. Otis, whose assistance is gratefully acknowledged.

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Extracellular Deoxyribonucleic Acid of Bacteria and a Deoxyribonuclease Inhibitor

The slime layer of bacterial cells is an extracellular accumulation of viscous material, most commonly composed of some high-molecular-weight polysaccharide. Smithies and Gibbons (*1*) found that the slime layers of various halophilic bacteria contain deoxyribonucleic acid (DNA). The slime could be dispersed by the action of pancreatic deoxyribonuclease (DNase), with the liberation of soluble DNA-split products, without affecting cellular viability. More recently, the viscosity of the ropy sediments produced by a number of nonhalophilic bacteria has been shown to depend likewise on the presence of polymerized extracellular DNA (*2*). Investigations of *Brucella* (*3*) indicate the presence in certain cultures of extracellular DNA that is sufficiently polymerized to produce a fibrous precipitate in ethanol (*4*). Because of the interest attaching to the extracellular accumulation of a supposedly intracellular gene-associated type of material, DNA was isolated from cultures of various bacteria by methods designed to leave the cells intact. In further investigations (*5*), a mechanism leading to the accumulation of DNA slime was found to be the production of extracellular ribonucleic acid (RNA), which is capable of inhibiting DNase action.

Slime-layer DNA preparations were obtained from *Micrococcus citreus, M. pyogenes* var. *aureus* (three strains), *Alcaligenes faecalis* (two strains), *A. viscosus,* and three strains originating as laboratory contaminants, which were notable for their massive accumulation of slime. Of these, strain B is a species of *Pseudomonas* and strains C and E appear to be members of *Flavobacterium. Micrococcus pyogenes* var. *aureus,* which is pathogenic for human beings, grows readily in mediums containing 7.5-percent NaCl (1.3*M*) but usually is not considered to be a halophile. The other bacteria were considerably less salt tolerant, their growth being inhibited by 1.25*M* NaCl, and maximum growth was obtained only in broth containing less than 0.25 to 0.5*M* NaCl.

Micrococcus pyogenes var. *aureus,* which produces large quantities of an extracellular DNase under certain conditions (*6*), formed visible slime in young nonaerated brain-heart infusion (Difco) cultures; more massive accumulations were obtained when 1*M* NaCl was present or when the broth was buffered at *p*H 6.0 (succinate, 0.05*M*)—procedures designed to minimize DNase action. The other bacteria formed DNA slime under various conditions; commonly, a peptone-yeast extract broth was used. Cultures were harvested, usually after 2 to 4 days, by aspirating off the nonviscous broth from the mass of slime-covered cells. The slime was stirred with 0.41-percent sodium dodecyl sulfate for 3 hours, and NaCl was added (final concentration 1*M*) with further stirring. The viscous mixture was centrifuged and cells, which were revealed to be intact by microscopic examinations, were discarded. Nucleic acid was precipitated from the supernatant by addition of ethanol. Yields at this stage from strains B and C were commonly 200 mg of crude, dry product (containing 40 to 45-percent DNA) per liter of broth culture. One or two additional steps of purification with detergent were carried out (*7*).

Curves of the ultraviolet absorption spectra for solutions of each slime-layer preparation were quite similar to the curve obtained using thymus DNA; maxima for all occurred between 256 and 259 mμ. Comparisons of deoxyribose (*8*) and phosphorus values indicated that, with preparations from *Micrococcus citreus, Alcaligenes faecalis,* and strain B, more than 95 percent of the nucleic acid was DNA. The other preparations, however, showed considerably higher proportions of RNA (*9*). Furthermore, ratios of phosphorus to dry weight indicated that about half of each sample consisted of phosphorus-free ultraviolet-nonabsorbing material, believed to have been mainly polysaccharide (*9*).

Although the microbial preparations were not pure DNA, the viscosity of the solutions was practically the same as that of solutions of thymus DNA (*7*) having equivalent deoxyribose (Dische reaction, *8*) content. By means of small Ostwald-type viscometers (*10, 11*), determinations were made of the rates of viscosity reduction by crystalline pancreatic DNase (Worthington) of each of the slime-layer preparations and of thymus DNA. The reaction mixtures at 37°C contained 10^{-6} mg/ml of pancreatic DNase, 0.8 to 1.2 mg/ml of DNA, and imidazole buffer at *p*H 7.3, with 0.025*M* $MgCl_2$. The rates of depolymerization were essentially the same for all the samples (and their viscosities were reduced to that of water in each case, except *Alcaligenes viscosus*), thus verifying the presence of highly polymerized DNA.

Such DNA could not accumulate in the presence of active depolymerizing enzyme. Investigations of strain B showed that, under certain conditions, broth cultures may evidence depolymerizing activity when tested against thymus DNA but little or no activity against strain B DNA. This suggested that strain B and possibly the other bacteria also, may produce a deoxyribonuclease inhibitor (*11, 12*).

Table 1 shows the effects of treating strain B DNA with crystalline ribonuclease (RNase, Worthington), which was found to be free of DNase activity. The DNase of strain B, which resembles pancreatic DNase in being activated by Mg at *p*H 7.3, was obtained from cells (shaken with ballotini in a Mickle disintegrator) of a young culture. Using a fresh dilution (1/20 in 0.1-percent gelatin) of this disintegrated-cell preparation each time, parallel viscometric tests were carried out with DNA from two sources.

Table 1. Effect of ribonuclease on susceptibility of a preparation of slime-layer DNA to the subsequent action of DNase.

RNase treatment of strain B DNA (before adding DNase)		Activity (units) of strain B DNase against	
RNase (mg/ml)	Time incubated (min)	Strain B DNA	Thymus DNA (control)
No RNase treatment		1.3	12
5×10^{-4}	1	10	13
	120	12	12
1×10^{-5}	2	7	13
1×10^{-6}	7	6	13
1×10^{-7}	2	1	12
	65	2	12
	2880	6	13