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# A STUDY ON THE BIOCHEMISTRY OF ALARM SUBSTANCES IN FISH

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## ABSTRACT

Two cyprinid fishes, *Clinostomus funduloides* and *Notropis cornutus* were tested with naturally occurring substances including some well-known biogenic amines. Behavioral responses to histamine were similar to those observed in previous tests with natural alarm substance extracts. A response threshold was obtained at 0.01 ppm. Spectrophotofluorometric emission spectra also indicated that the natural alarm substance known to exist in many species of fish may be a ringed or double ringed compound.

## INTRODUCTION

Alarm substances and fright reactions in fishes have been studied by several workers (Von Frisch, 1941; Schutz, 1956; Pfeiffer, 1960, 1962, 1963, 1966, 1967; Reed, 1969). The alarm substance has been shown to be present in the skin of several families and at least three orders of fishes (Pfeiffer, 1967; Reed, 1969). Pfeiffer (1960) suggested that the alarm substance is contained in club cells present in the epidermis of fish skin and that this substance can only be released by damage to the skin. The substance is water soluble and is perceived by olfaction (Von Frisch, 1941b). Purrman (1947) stated that ichthyopterin, which is contained in the skin, was identical with the alarm substance but Schutz (1956) found that synthetic ichthyopterin did not produce a fright reaction although it had certain chemical properties similar to those of the natural alarm substances.

Huttel (1941) suggested that the alarm substance was a purine or pterin-like compound and showed that the substance is not volatile, although extremely soluble in water.

To provide additional information concerning the chemical nature of the alarm substance in fishes, a study was initiated using several qualitative and quantitative biochemical techniques.

## METHODS AND MATERIALS

The source of the natural alarm substances in this study was two cyprinid fishes, *Notropis cornutus* (Mitchill), the common shiner, and *Clinostomus funduloides* Girard, the rosyside dace. Both species were tested to determine if an alarm substance were present in the skin. Testing consisted of a series of behavioral experiments using skin extract as described by Von Frisch (1941a, 1941b) and modified by Reed (1969).

In addition to the natural skin extract, several naturally-occurring compounds including some biogenic amines were tested. They included norepinephrine, acetylcholine and histamine. These compounds have all been suggested to function as neurotransmitter agents in most animals.

These compounds plus gamma amino butyric acid and the crude skin extract were also studied for chemical properties using an Aminco-Bowman Spectrophotofluorometer, paper chromatography, gel column separation, and a Gilson high voltage electrophoresis apparatus.

The results of the behavioral experiments were compared to the data from the biochemical analyses to determine the relationships of the chemicals tested to the natural skin extract.

## RESULTS AND DISCUSSION

The preliminary behavioral testing showed that a positive fright reaction with the two cyprinids was elicited in response to perfusion with skin extracts. In addition, a positive response was elicited when histamine was the test substance at a concentration of 0.01 ppm. The positive response to histamine was weaker than with the natural alarm substance. Higher concentrations of histamine (0.1 and 1.0 ppm) did not elicit stronger fright reactions.

Norepinephrine and acetylchloine did not cause a positive fright reaction at concentrations of 0.01 and 0.1 ppm.

Spectrophotofluorometric analyses showed a similarity in several peaks of the emission spectra of the skin extract and histamine, acetylcholine and GABA when tested at the same excitation wavelength. Similar results were obtained after increasing the resolution of the technique by altering the wavelength of excitation.

These compounds are somewhat similar in structure, being low molecular weight substances with terminal amine groups. Both GABA and acetylcholine have a carboxyl group present. GABA has as yet not been used in behavioral experiments.

Preliminary results using high voltage electrophoresis have been encouraging in that the various low molecular weight molecules have undergone rapid and distinct separation. However, precise identification of the natural amine compounds contained in skin extracts has not been possible owing to the extremely low concentrations of these substances. Greater clarification is possible by combining gel columm extraction with perfusion of the separated substances. Initial work in this area is proving to be helpful in the isolation and purification of the alarm substance. Thus far gel column separation seems to be the most feasible method for obtaining sufficient quantities and concentrations of the alarm substance.

Like epinephrine, norepinephrine, acetylcholine, histamine, and gamma amino butyric acid the alarm substance may function as, or be similar to some neurotransmitter agents in fish. Ecologically it is probable that the alarm substance functions in species preservation.

From the data gathered thus far it is possible that the alarm substance could be a short-chained molecule, having an aliphatic rather than an aromatic ring, of low molecular weight, and highly soluble in water. Because various species of

fish show a different degree of response to intraspecific extracts than to extracts of other species, it is possible that the alarm substance may have a polypeptide-like structure. If this were the case, the different degrees of response could be attributed to the addition or deletion of different amino acids in the chain. Such a compound could consist of an active site, which elicits the actual fright reaction, and various functional groups (probably species dependent), located at key points in the chain which would somewhat alter the environment of the active site. This alteration could account for the different degrees of response from different species.

The work of Pfeiffer (1966b) involving crosses between *Astyanax mexicanus* (Filippi) and *Anoptichthys antobius* points toward genetic control of the fright reaction and possible coding for the synthesis of the alarm substance.

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