EVIDENCES OF NEW ICHTHYOINTOXICATIVE PHENOMENA IN *GYMNODINIUM BREVE* RED TIDES[1]

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ABSTRACT

A red tide caused by the toxic dinoflagellate *Gymnodinium breve* Davis occurred intermittently along the west coast of peninsular Florida from October 1973 through June 1974. Distress behavior of fishes in the red tide area was observed and 129 severely distressed or freshly dead specimens were collected and subjected to immediate necroptic examination. The 16 consistently observed pathologies suggest that many fishes die in red tides from chronic tissue damage rather than by previously recognized neurointoxication. Some species seem to succumb to neurointoxication under the same conditions that produce lethal hemopathy and histopathology in others. The observed sign complexes are indicative of dehydration, hemolysis, and interferences in the blood clotting mechanisms.

INTRODUCTION

Substantial red tides caused by blooms of the unarmored dinoflagellate *Gymnodinium breve* Davis occur periodically along the west coast of Florida (1, 2, 3). In addition to causing toxicity in shellfish, *G. breve* red tides directly kill large numbers of fish and some marine invertebrates, produce a highly irritating aerosol in coastal areas, and can rarely cause contact dermatitis (4, 5, 6, 7, 8, 9). As a result of these effects, such red tides have serious adverse economic impact, particularly on the tourist industry, in addition to being a public health problem.

Considerable scientific study has been made of *G. breve* red tides, particularly since the discovery and description of the causative dinoflagellate by Davis in 1948. Early laboratory studies indicated that fish were killed by a neurotoxin as evidenced by speed of action, agonal behavior, and lack of other patent signs or lesions (10, 11). These observations were repeatedly confirmed by numerous experimental exposures of fishes to natural and cultured *G. breve*, to cell-free filtrates, and to extracted and fractionated toxins (11, 12, 13, 14, 15). Consequently, *G. breve* red tides became accepted as a neurointoxicative phenomenon.

Detailed studies of toxins extracted from *G. breve* in recent years have provided evidences of other biological activity. In addition to potent neurotoxic activity, *in vitro* hemolytic activity was detected in crude toxin extracts (16, 17, 18, 19, 20, 21, 22, 23) and in a purified non-neurotoxic fraction (15). Hemolytic activity was considered mild until Kim, Linton, and Martin (23) tested fish blood (mullet) in addition to the mammalian blood (usually rabbit) utilized by other workers. They found the piscine blood to be some 300 times more sensitive. They suggested that hemolytic activity could be a factor in red tide fish kills.

Doig and Martin (24) further discovered mild anticoagulant activity in toxin extracted from a natural red tide. Trieff *et al.* (25) isolated five toxins from *G. breve* cultures, one of which was a heretofore unreported, severe respiratory

irritant upon either intraperitoneal injection or inhalation. All five of their toxins had neurotoxic activity and one was also hemolytic.

Despite the findings of additional biological activities, the neurotoxic activity of *G. breve* has still been generally considered to be the only consequential ichthyointoxicative mechanism. This assumption was supported by the few reports of premortal fish behavior in natural red tides (5, 26, 27) which indicated, as laboratory studies had suggested, that death usually came rapidly upon exposure to *G. breve* red tide.

Beginning in October 1973, we collected dead and dying fishes and other organisms from red tide areas and subjected them to detailed pathological analyses to determine mechanisms of death. Preliminary results were presented by Quick and Henderson (28). Further results and interpretations are presented herein. These studies are the first pathological examinations made of red tide exposed animals from the field or laboratory since the discovery of the causative agent over 25 years ago. In fact, a few brief comments by Taylor (29) concerning darkened gills and slow blood clotting in dissected fishes seem to represent the only prior published observation of organic changes caused by red tide.

METHODS AND MATERIALS

Beginning in October 1973, a red tide developed offshore of the west central coast of Florida in the Gulf of Mexico and moved shoreward. After a short absence, it reappeared in January 1974 and moved inshore along some 270 linear km (168 miles) of coastline and penetrated Boca Ciega Bay, Sarasota Bay, Tampa Bay, and Charlotte Harbor. Severe, widespread fish kills resulted along with respiratory irritation and toxic shellfish.

In late October 1973, we began collecting distressed or freshly dead fishes from the red tide areas. Collections were made with aid of SCUBA, with nets, and/or by hand. Every effort was made to observe any distress behavior. Water samples were collected at or near fish sampling locations and analyzed quantitatively for *G. breve* (cell counts) in addition to the usual hydrographic parameters.

Specimens and samples were rushed without refrigeration back to our main laboratory in St. Petersburg. Most analyses were begun within 45 minutes of collection or death and none after more than 6 hours. Fishes were subjected to a detailed necroptic analysis. After initial observations and measurements (color, slime, length, weight, etc.) blood samples were taken from one or more of the sinuses or major veins just afferent to the heart. Blood was subjected to standard hematological analyses including cell counts, clotting, and chemistry. Careful necroptic dissection followed. Tissue samples were excised and fixed for later histological technique processing and for preparation for electron microscopy. Procedures are detailed in Quick and Henderson (28).

Following the 1973-74 red tide, several others occurred from which additional specimens were collected and examined. In August 1974, a red tide

developed in the Gulf off the Tampa Bay area and moved shoreward. Many sharks appeared inshore, apparently driven ahead of the advancing dinoflagellate bloom. These sharks, principally young spinner and black tip species (*Carcharinus maculipinnis* and *C. limbarus*), became progressively lethargic and died. Several specimens were netted in distress and subjected to necropsy. As is usual with sharks, none floated after death and the mortality was relatively inobvious.

The following month, red tide appeared in north Florida near Panama City and moved westward for several weeks, finally subsiding near Pensacola, Florida. Widespread fish kills occurred and irritating aerosols were severe. Several cusk eels (*Ophidion holbrooki*) were collected in *rigor mortis* and necropsied.

So far, 129 fishes of 15 species and genera representing 13 families, 8 orders, and 2 classes have been subjected to detailed pathological examination.

RESULTS

Analyses are still incomplete, but initial findings already indicate that *G. breve* red tide ichthyointoxication may be substantially different from the processes previously thought to occur.

Sixteen identifiable pathologies occur repeatedly in red tide exposed fishes and seem to be due, directly or indirectly, to *G. breve* toxins: 1) anoxic premortal distress behavior, 2) cyanemia and branchial cyanosis, 3) severe achromic normoblastosis, 4) moderate to severe normochromic normacytic anemia, 5) marked hemal hyperviscosity, 6) increased whole blood clotting time (WBCT), incipient and total, 7) increased packed cell volume (PCV), 8) increased total blood count (TBC), 9) increased mean cell volume (MCV), 10) thrombocytopenia, 11) leucocytopenia, 12) plasma debris, 13) manifest plasma hemoglobin, 14) absent or reduced recent feeding, 15) splenomegally, and 16) excessive hepatic vascular endothelial hemosiderin deposition. Each of these pathologies is described in some detail in Quick and Henderson (28).

Observed pathologies tended to vary somewhat from one individual specimen to the next, particularly quantitatively. Since all cases were natural exposures, we can only infer the level of toxin(s) each may have encountered from our knowledge of *G. breve* concentrations in the collection area at and before the time of collection. One would, therefore, expect the effects on the fishes to differ somewhat from place to place and time to time in different collections. Some differences were seen, however, between specimens of the same species from the same collection and presumably from the same school. Since these individuals probably had the same red tide exposure history, we must conclude that there is some variation in individual response to *G. breve* under similar conditions. This variation may be due to individual genetic or physiological postures.

A much greater diversity of pathological responses was seen between species than between individuals of a single species. In fact, the two species for which we had the most cases, the striped mullet (*Mugil cephalus*) and the ladyfish (*Elops saurus*) showed almost completely opposite responses to a red tide intoxication.

CONCLUSIONS

As we said earlier, acute, non species specific neurointoxication is the rule in laboratory studies of *G. breve* toxin and has been inferred from limited field observations. The results of our studies of naturally exposed fishes, however, lead us to propose two additional mechanisms of death in red tides: 1) chronic neurointoxication and 2) chronic lethal hemopathy. Unlike the acute neurointoxication which seems to result in death of any fish exposed to sufficient toxin, the two chronic intoxications seem to be species specific, the ladyfish being a good example of the former and mullet of the latter. Many fishes show elements of both.

In chronic neurointoxication, exposed fishes seem to act almost normally during many hours or days of exposure to red tide. A neuromuscular crisis then occurs causing extreme, uncontrolled, but somewhat coordinated, hyperactivity. Rapid swimming in large circles and leaping from the water is typical. This degenerates to an uncoordinated vibrating and tumbling and death ensues rapidly, usually in 15 to 30 seconds after the beginning of the agonal distress period. Terminal cardiac arrest was directly observed in small transparent anchovies (*Anchoa mitchelli*). At necropsy, chronically neurointoxicated fishes show little pathology aside from slight precipate hemolysis as evidenced by corpuscular ghosts, plasma debris, and patent hemoglobinemia, and frequent cerebrovascular congestion. The hemolysis is consistent with that seen in cases of lactic acid buildup in the blood due to capture stress and struggle (30). It is therefore likely that it results from the fatal agony rather than directly from red tide effects. It may be significant that ladyfish and anchovies seem to be exceptionally susceptible to lethal hemopathic acidosis from capture stress under non red tide conditions.

Individual fishes of the same species seem to vary in their sensitivity to the red tide toxins. Typically, in a large school under continuous exposure, individual fish randomly but suddenly enter the premortal frenzy and expire. The majority continue to act normally until each, in turn, is affected.

In chronic hemopathic intoxication, exposed fishes seem to show a progressive general hypoesthesia, particularly ocular hypoesthesia and hypacusis, and hypokinesia. Balance and coordination do not seem to be affected and once a fright response is elicited, it appears normal.

As before, premortal distress seems to overtake individual fish suddenly, frequently following a fright response or other exertion. Unlike the previous hyperactivity, fishes in terminal hemopathic distress undergo a slightly

hypoactive, anoxic behavior mimicking the surface breathing and gulping seen in fishes immersed in oxygen deficient waters. Progressive weakening follows and the fish begin to sink for short periods before swimming back to the surface. These periods of sinking toward the bottom increase in length as the time at the surface decreases. Swimming becomes increasingly encumbered as coordination is lost and death comes uneventfully. Sometimes, there is a moment of uncoordinated hyperactivity just premortal. Time from initiation of terminal distress to death was usually 15 to 30 minutes.

At necropsy, these fish typically showed most if not all of the 16 pathologies listed previously. The fish apparently ate little or nothing in their hypoesthetic-hypokinetic state.

Hemolysis also seems to occur chronically during this period of almost asymptomatic exposure to red tide. The finding of well developed normacytic anemia with replacement normoblastosis is evidence of selective hemocatheresis of some duration. The splenomegally and pronounced hepatic hemosiderosis, common results of such chronic hemoclasis, further support this interpretation.

However, findings of patent plasma hemoglobin, plasma debris, and corpuscular ghosts are indicative of an acute hemolytic crisis. The degree of hemoglobinemia, often approaching the corpuscular hemoglobin, far exceeds that found in cases of distress mediated lacticemia. It would seem that some unusual rapid hemolysis occurs during terminal distress in addition to the long term preexisting chronic hemolysis. The observation of the rapid darkening of the gills of fishes upon laboratory exposure to *G. breve* (Doig, pers. comm.) and of the ears and feet of injected mice (Trieff, pers. comm.) may be a manifestation of similar acute hemoglobinemia in these animals. The observed cyanemia could have resulted from the hemoglobinemia except that it was nonreversible in air.

The markedly elevated hematocrit (PCV) is indicative of dehydration as is the increased TBC. Hyperviscosity would also be expected under these conditions, although the observed level greatly exceeds that seen in previous cases of dehydration. The indicated level of hemoconcentration is much greater than that resulting from periods of exercise (31). Teleosts can be quite susceptible to dehydration due to their dependence on active transport mechanisms in gills to maintain their hypotonicity relative to sea water. Thus, agents that disrupt this or associated physiological mechanisms can cause rapid dehydration. Our hypothesis of dehydration of teleosts by red tide is further supported by the fact that elasmobranchs, normally nearly isotonic to sea water, showed little or no increase in hematocrit, TBC, or viscosity. Although there were no direct indications of the duration of the hemoconcentration, experience suggests that such a severely increased viscosity (whether or not due to dehydration) was sufficiently pronounced to have been rapidly lethal by spontaneous capillary hemostasis. The hepatic vasoconstriction due to endothelial hemosiderosis would be expected to aggravate this mechanism.

The markedly increased incipient WBCT and similarly increased or infinite total WBCT indicate severe disruption of the normal clotting mechanisms. The frequent findings of polymerized short fibrin fibers (soft clot) suspended in the serum suggest some diffuse intravascular coagulation may be active. These fibers did not anastomose nor contract upon standing and, in most cases were not adhesive to erythrocytes. This presence of suspended fibrin fibers may act synergistically with the increased PCV to produce the observed hemal hyperviscosity. The thrombocytopenia is further evidence of clotting abnormalities. The few thrombocytes present did seem to show normal coagulative activity, however.

These proposed intoxicative mechanisms plus other observations including pronounced cyanemia invite the question of what is the actual cause of death in fishes that have hemopathic response to red tide. At least four of the conditions seem to be potentially lethal: dehydration, hemal hyperviscosity, cyanemia, and hemocatheresis. Some clue may be provided by the anoxic premortal behavior of affected fishes. It would be expected that replacement of erythrocytes by achromic normoblasts would greatly decrease the oxygen transport capacity of the blood. The irreversible cyanemia is further evidence of loss of availability of hemoglobin for oxygen transport. Any decrease in blood flow due to hyperviscosity would further aggravate this situation. It may thus be that fish susceptible to red tide hemopathy succumb by histologic anoxia even though sufficient oxygen may be present in the surrounding water. Such a mechanism also suggests a synergism with depressed sea water oxygen concentrations. Several unusual fish kills associated with red tides, such as those reported on offshore reefs by Smith (32) may have been accentuated or caused by such a synergism.

This work is still continuing with particular emphasis on clarifying some of the processes and mechanisms hypothesized in the foregoing section and to determine the significance of other conditions such as leucocytopenia and cyanemia that remain unexplained. Further studies will be made through laboratory exposures of selected marine animals to controlled, quantitated *G. breve* cultures.

DIAGNOSTIC APPLICATIONS

One of the important and most difficult activities of marine environmental biologists is the determination of cause in fish kills and other mortalities, epizootics, and epiphytotics. The finding of an identifiable, unique sign complex in at least some species of fish commonly killed in *G. breve* red tides provides a diagnostic tool of considerable value.

Heretofore, diagnosis of red tide has only been possible if a properly collected, carefully transported, fresh, water sample was available for analysis. Now, at least with some species of fish, such a diagnosis can be made without water samples. Fortunately, the species that show the strongest hemopathic signs are also those that die earliest and most commonly in *G. breve* red tides.

Already, diagnosis of red tide has been made by examination of fish in several cases and later supported by water collections: in two mullet kills thought to have resulted from culls and losses from nets of fishermen; in an offshore kill of grunts in which the actual red tide was concealed below the thermocline; and in a shark kill in which low oxygen had been suspected. In a reverse situation where cusk eels were thought to have been killed by *G. breve* as confirmed by water samples, necroptic analysis indicated anoxia to be the primary cause of death. In one surprising case, freshwater goldfish showed a particularly well developed red tide sign complex. It appears from this and other evidence that the *G. breve* toxins must have been transported as aerosols from the nearby red tide affected bay to the pools containing the hypersensitive fish. In another case, an extensive, massive duck kill occurred in a red tide area. Analyses showed mild red tide hemopathy in the ducks. Other data, however, did not indicate the red tide to be causative of the bird mortality and the hemopathic syndrome was judged nonlethal.

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