

NATURE OF TOXINS

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**NATURAL TOXINS AND HUMAN PATHOGENS
IN THE MARINE ENVIRONMENT:
PROBLEMS AND RESEARCH NEEDS**

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Chapter 1

CIGUATERA SEAFOOD POISONING: A CIRCUMTROPICAL FISHERIES PROBLEM

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Introduction

There are several human illnesses associated with the consumption of particular seafoods that, by virtue of the publicity they have received, have a far-reaching, negative impact on the entire fishing industry. One such illness that seafood consumers in tropical and subtropical areas of the world have long recognized is the very unpleasant and potentially dangerous illness called "ciguatera." This particular poisoning is acquired by the ingestion of fish that accumulate through their diet a heat-stable, currently undetectable toxin named ciguatoxin. The fish may be any one of a wide variety of tropical or subtropical reef fish, including those of commercial and recreational value, such as snapper, grouper, barracuda, and amberjack. In addition to affecting public health directly, ciguatera can have a dramatic impact on the economy of an entire regional fishing industry through the local public's concern and apprehension regarding the safety of all seafoods. And while the illness is usually limited to the warmer climates of the world, ciguatera has been reported with increasing frequency in temperate climates due to the return of tourists afflicted with ciguatera, as well as intensified interstate commerce of tropical reef fish. Only recently have members of the northern U.S. fishing industry and physicians practicing in temperate climates become aware of this illness and of its dramatic impact on tropical fisheries. For more detailed information on ciguatera, the reader should consult the recent review article by Withers (1982).

Impact of Ciguatera on Human Health

Ciguatera is a serious health and socio-economic problem, involving the fisheries in tropical and semitropical areas of the world (Banner, 1976; Halstead, 1978; Helfrich and Banner, 1968). One difficulty in assessing the health impact of ciguatera is the absence of diagnostic means to identify and evaluate clinical cases. Reasonable estimates suggest that each year between 10,000 and 50,000 individuals are afflicted worldwide. Documented annual morbidity statistics for specific geographical areas range from approximately 0.5 cases per 1,000 population in low incidence areas such as Florida (Lawrence, et al., 1980) to 27 cases per 1,000 population in high incidence areas such as the U.S. Virgin Islands (McMillan, Grande and Hoffman, 1980). Other

areas that experience frequent episodes of ciguatera include Puerto Rico, Fiji, American Samoa, French Polynesia, the Queensland coast of Australia and the British Virgin Islands (Bagnis, 1980; Dawson, 1977; Lewis, 1981). Fortunately, the mortality is less than 1 percent of reported cases (Lewes, 1981; Bagnis, Kuberski and Laugier, 1979). Even though ciguatera is not a reportable disease, the Center for Disease Control (Atlanta, GA) has listed ciguatera as the most frequently reported foodborne illness of chemical origin (Hughes, 1979).

Symptoms of ciguatera involve gastrointestinal, neurological, and cardiovascular systems, leading in extreme cases to death by respiratory failure and/or severe dehydration (Lawrence, et al., 1980; Bagnis, 1973). Diagnosis of ciguatera is based on clinical observation and patient history; there is no confirming laboratory test. An episode of ciguatera intoxication does not appear to confer immunity; in fact, an enhanced reaction to the pharmacological effects of the toxin may occur with successive exposures. The unusual neurological symptoms of paresthesia and temperature reversal are considered diagnostic hallmarks, and these often recur or are exacerbated following physical duress or the consumption of an alcoholic beverage. Unfortunately, there is no "cure" for ciguatera and management of patients is based solely on supportive measures.

Despite the absence of reliable morbidity statistics, it is immediately apparent that the illness is of widespread concern in both the Caribbean and South Pacific areas, where it presents a serious health hazard and limits the utility of fish resources in island regions whose populace depends on reef fish as an important source of protein.

Impact of Ciguatera on the Development, Growth and Stability of the Fishing Industry

Because of the publicity associated with the devastating long-term effects of this illness, ciguatera has had a dramatic impact on the tropical fishing industry and its associated trades. Public apprehension of seafood safety that borders on hysteria was dramatically illustrated by the public's reaction to a ciguatera case reported by the banner headline "Death at the Dinner Table" in the San Juan Star's Sunday Supplement (October 4, 1981). Unfortunately, ciguatera cases are often reported with melodramatic flair, which generates panic and forces health officials to confiscate restaurateurs' entire stock of fish, even when such fish cannot reliably be incriminated in the illness. In addition, many of those afflicted are often associated with the local fishing industry and therefore do not report an incident that could have a serious impact on their livelihood. Reports of ciguatera are almost always followed by an immediate and drastic reduction in the sales of seafoods. Additional economic loss to the seafood industry occurs when afflicted individuals refuse to eat fish due to personal aversion or clinical admonition for many months or years after the intoxication. The greatest constraint to the industry occurs when reports of ciguatera have led to the ban on sale of selected fish species (barracuda in Dade

County and Puerto Rico, large amberjack in Hawaii, etc.). Estimates of losses to the industry have not been adequately determined, but it is reasonable to approximate the annual losses at \$10,000,000 to the Florida/Caribbean industry.

Any effort to start a fishing industry, or to expand a marginal operation in the tropics, immediately encounters the problem of ciguatera. And while many islanders often regard ciguatera as an expected nuisance, or the price paid as a consequence of eating local reef fish, the potential threat of ciguatera continues to be a major hurdle in fisheries development, from both a public health perspective and from litigation issues which often follow (Hughes, 1979). Recent court cases of ciguatera, and pending litigation, have forced seafood wholesalers to pay a higher price for liability insurance. As reported by Dammann (1969), the danger of ciguatera poison remains one of the major deterrents to efficient and widespread marketing of most tropical species of shallow-water food fish. Until the health hazards of ciguatera can be resolved, there appears to be little possibility of developing or improving the present inshore fisheries in ciguatera-endemic tropical areas.

Laboratory Production of Ciguatoxin

An inherent difficulty in carrying out laboratory-based research on ciguatera is the ability to acquire quantities of toxin(s) sufficient to conduct the chemical and biological studies needed to advance our understanding of the illness. Although readily capable of causing serious human illness, ciguatoxin fish contain extremely small amounts of the poison. Its isolation and purification was accomplished by Scheuer, et al. (1967) 15 years ago, when 2,000 pounds of liver tissue from toxic moray eels yielded only 1.5 mg, or 0.00006 ounce, of toxin (intestines, gonads and liver were found to harbor fifty to two hundred times more ciguatoxin than muscle). Extraction of ciguatoxin from clinically-incriminated fish flesh yielded ciguatoxin but in such small quantities as to make this source impractical for chemical studies.

Recently, the dinoflagellate Gambierdiscus toxicus has been implicated as the microorganism that synthesizes ciguatoxin and initiates its transmission through the food web (Adachi and Fukuyo, 1979; Yasumoto, 1980; Withers, 1981). This finding raised hopes that laboratory culturing of the dinoflagellate could yield significant quantities of ciguatoxin. Unfortunately, this optimism has been tempered, since the presence of ciguatoxin has not been confirmed in laboratory cultured cells as distinct from wild cells (Helfrich and Banner, 1968). Cultured cells do produce maitotoxin, a water soluble toxin. Both maitotoxin and scaritoxin are secondary toxins which frequently coexist with ciguatoxin in suspect fish; their involvement in ciguatera, however, needs to be resolved. Since wild G. toxicus cells collected from their natural habitat yielded substantial amounts of ciguatoxin-like material (Helfrich and Banner, 1968), it is reasonable to assume that physiochemical parameters may be found that will result in production of significant levels of toxin within the laboratory setting. Hopefully, such studies may ulti-

mately result in the use of cultured cells for obtaining ciguatoxin. Until these culture conditions have been ascertained, however, researchers will have to rely on large-scale extraction of suspect fish as their source of ciguatoxin.

Chemical Research

A complete description of the molecular structure of ciguatoxin, the principal lipid toxin responsible for ciguatera, may be forthcoming. Only recently has ciguatoxin been crystallized; unfortunately, the crystals are too small for structural elucidation by X-ray diffraction (Hokama, Kimura and Miyahara, 1980). One milligram of crystalline toxin constitutes the entire supply worldwide and more is needed to enhance crystal size in order to resolve its structure. Since the parameters for crystal nuclei formation are not known, "trial and error" experiments must be conducted to find optimum conditions for maximum crystal size. With a limited toxin supply, only one or two experiments can be conducted at a time and each experiment takes several weeks. Furthermore, no crystallographer has succeeded to date in determining the molecular structure of any molecule of the size of ciguatoxin (M.W. = 1,112) by direct methods. A crystalline heavy atom derivative of ciguatoxin must therefore be prepared if successful X-ray crystallography is to be realized. Obviously, the possibility of obtaining a molecular structure for ciguatoxin will be much improved once additional quantities of the toxin become available.

In addition to elucidating the structure of ciguatoxin, chemical studies are required in order to identify several other toxins often found in association with ciguatoxic fish. Preliminary evidence suggests that dinoflagellates, in general, and G. toxicus, in particular, produce a variety of toxic material; only by carefully defining these materials on the basis of their chemical composition will their relationship, if any, to ciguatoxin be determined. Furthermore, an accurate chemical definition of the responsible agent is mandatory, if similarities or differences in the illness among geographic regions (i.e., Pacific vs. Caribbean) are to be documented. Unfortunately, an inadequate chemical description of the extracted "toxin(s)" used in many former studies makes it virtually impossible to interrelate data. A thorough knowledge of the toxin's structure is a key element in many other endeavors, including the development of a practical field test for ciguatoxin, the analysis of its toxicological effects, the development of specific antidotal therapy, and the verification of the microbial source(s) of the toxin.

Dinoflagellate Research

From all available evidence, it is almost certain that the toxin(s) responsible for ciguatera have their origin in one or more benthic dinoflagellate species. The range of species involved, and the environmental conditions that favor toxin synthesis, are unknown. Obviously, a thorough knowledge of the incriminated dinoflagellates will greatly enhance

our understanding of the vagaries of the illness, including the mechanism for transmitting the toxin through the food web. In general, research investigations involving dinoflagellates comprise several inter-related studies. (i) Although G. toxicus is known to produce ciguatoxin in its natural habitat, the production of this toxin has not been achieved in laboratory cultures. It is necessary, therefore, to determine growth phases and environmental conditions which support the biosynthesis of ciguatoxin in unialgal and axenic cultures. (ii) Since other dinoflagellates may synthesize ciguatoxin, it is important to isolate and culture other species of dinoflagellates which occur in regions known to harbor ciguatoxic fish and to test for toxin(s) production. (iii) To date, various species of dinoflagellates have been shown to produce toxins, some of which exhibit properties similar to ciguatoxin, maitotoxin, okadaic acid or saxitoxin. However, most of these toxins have not been purified or well defined chemically or biologically, and a great deal of effort must be applied if these results are to have meaning.

Underlying all research endeavors involving the marine dinoflagellates is an attempt to understand the process by which dinoflagellates introduce the toxin(s) into the ecosystem. Once the mechanism is known, it may be possible to predict and/or monitor localized marine areas as reservoirs for ciguatoxic fish on the basis of the prevailing environmental conditions that favor dinoflagellate growth and toxin production.

Ciguatoxin Detection and Analysis

Several options have been considered for minimizing the threat of ciguatera. These options range from simply avoiding those fish often incriminated in the illness, to seeding suspected "ciguatoxic hot spots" with mutant dinoflagellates that are defective in toxin biosynthesis. The comparatively large number of fish species that have been involved with ciguatera obviates a simple avoidance procedure for resolving the problem, and the technology for selecting mutant dinoflagellates and for altering conditions that would permit the mutant clones to overgrow the natural population has not been developed. The option that has received the greatest attention involves the development of a practical "market place" test for the presence of ciguatoxin, with a view to removing suspect fish from the stream of commerce.

Although this latter option appears most practical, the only reliable methods to date for detection of ciguatoxin (or any of the associated toxins) is animal bioassay, for which the mouse has become the favored animal model. Unfortunately, mice are relatively refractory to the effects of ciguatoxin and death, the end point of the bioassay, is not a classical response in humans. Nevertheless, this animal bioassay, although labor intensive, expensive and of questionable sensitivity, is currently the only acceptable basis for determining the biological activity of ciguatoxin preparations.

Without knowledge of the chemical structure of ciguatoxin, the possibility of developing a test to identify the toxin by classical

chemical means is unlikely. Because of the minute amount of toxin in fish flesh, and the lack of sensitivity intrinsic to many of the traditional chemical detection systems, sensitive immunological methods need to be pursued, with a goal of developing a ciguatoxin-specific reagent (anti-ciguatoxin antibodies). Although convincing evidence for the existence of this immunological reagent has not appeared, it is believed that this approach is most likely to provide the basis for future ciguatoxin assays.

Toxicology Studies

Ciguatera is an exceedingly unpleasant illness in man, with serious gastrointestinal and neurological disturbances occasionally culminating in death (Lawrence, et al., 1980). Unfortunately, there is as yet no medical treatment for ciguatera beyond measures designed to relieve some of the early symptoms. If the illness runs its typical course, gastroenteritis develops several hours after ingestion of a ciguatoxic fish and may last for several days, followed by a generalized weakness. Neurological symptoms, particularly paresthesia (a sensation of prickling or tingling of the skin that has no objective cause) may last from two days to several weeks (or longer). In severe cases, death results from respiratory failure associated with paralysis of the respiratory musculature. If the illness is untreated, death may also result from severe dehydration secondary to vomiting and diarrhea.

Unfortunately, the molecular events that cause these symptoms are unknown. Progress towards elucidating the toxin's mode of action has been severely hampered by the lack of biologically and chemically defined toxic material for experimentation. The available information has been gained by utilizing crude toxin preparations from small individual fish samples associated with isolated episodes. The mode of operation has only permitted the most superficial endeavors. Elucidating the molecular events leading to neurological disturbance and/or death is a prerequisite to the development of a rational approach to a specific antidotal therapy. Careful and systematic studies with animal model systems, including in vitro organ transplants, are urgently needed, not only to develop effective treatment regimes but also to resolve some of the existing difficulties (i.e., sensitivity) present in the only ciguatoxin assay currently accepted, the mouse bioassay. In addition, detailed knowledge of the toxin's mode of action will contribute to our pool of biological methods, which can be used to differentiate ciguatoxin from other marine toxins, particularly those toxins which have been associated, directly or indirectly, with "ciguatera."

Summary

Ciguatera is a serious human illness resulting from the consumption of coral reef-associated fish that accumulate ciguatoxin through their diet. Thousands of inhabitants and tourists in tropical areas of the world are affected annually and the resulting publicity has impacted the sale of seafoods in tropical and subtropical regions. A variety of com-

mercially important reef fish may contain the currently undetectable, heat-stable toxin, thereby presenting a real threat to developing selected tropical fisheries. Research needs include the development of market-place detection methods for ciguatoxin in suspect fish, a thorough description of the molecular structure of the toxin, delineation and culturing of ciguatoxin-producing dinoflagellates, a comprehension of the mechanism by which the toxin is introduced into the ecosystem, and an understanding of the toxin's mode of action utilizing animal models.