

Book Title: HANDBOOK OF CLINICAL NEUROLOGY

Chapter Title: MARINE TOXINS

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## CLINICAL NEUROLOGY

### INTOXICATIONS OF THE NERVOUS SYSTEM: Marine Toxins

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#### **1. Introduction**

Throughout the world, marine toxins cause a variety of acute, subchronic and chronic diseases in humans, as well as disease in other mammals, fish and birds (Table 1) (Hughes & Merson 1976, Southcott 1979, Baden 1983, ILO 1984, Sakamoto et al, 1987, Halstead 1988). The diseases in humans range from acute neurologic diseases, such as Ciguatera and Paralytic Shellfish Poisoning, to chronic dementia as reported with domoic acid exposure. The marine toxins cause disease predominantly through the ingestion of contaminated fish and shellfish, although certain diseases are via skin contact and even with inhalation. Therefore, the food web and the biomagnification of these toxins through the marine food web play important roles in the transmission of the marine toxin disease. These marine toxins bioaccumulate in a range of intermediate marine hosts (ie. the transvectors) both shellfish and fish prior to contact with humans; often there are additional secondary transvectors with further bioaccumulation (such as carnivore fish eating contaminated herbivores).

**TABLE 1 HERE.**

In addition, most of the toxins are small, non-peptidic materials which are highly potent; exceedingly small quantities can lead to illness (ie. < 1 mg/kg body weight). For example, the marine toxin Ciguatoxin is estimated to be toxic to humans in a total body dose of 70 nanograms (ILO 1984, Miller 1991), and its co-occurring toxin Maitotoxin is estimated to be even more potent. The marine toxins are predominantly neurotoxins, although hemolytic substances have been identified. In general, the natural marine toxins are tasteless, odorless, and heat and acid stable, therefore normal screening and food preparation procedures will not prevent intoxication if the fish or shellfish is contaminated (ILO 1984, Sims 1987, Halstead 1988, Teitelbaum et al, 1990, Perl et al, 1990, Miller 1991).

The general source of these toxins, with the exception of tetrodotoxin in pufferfish and the blue green algae cyanophytes, are the dinoflagellates and diatoms. The dinoflagellates are

phylogenetically unique marine organisms. Dinoflagellates are single-celled algae-like biflagellated organisms dating back some 450 million years, with both prokaryotic and eukaryotic attributes. They can be benthic or pelagic organisms found throughout the marine world, especially in coral reefs and their surroundings (Levinton 1982, Winter et al, 1990, Miller 1991). Of the total known 2000 species of dinoflagellate, only about 20 species have been demonstrated to produce specific toxins (Steidinger & Baden 1984). Certain diatoms also can produce toxins under the proper environmental circumstances. Diatoms, like the dinoflagellates, are single-celled algae, but in this case they are not flagellated and are by definition enveloped by a silica wall or frustule. Diatoms, like the dinoflagellates, can exist in the water column or attached to solid surfaces.

Dinoflagellates are most infamous as the cause of "Red Tides," which are dense phytoplankton populations suddenly appearing, coloring the water red or brown. These Red Tides are often (but not always) associated with high concentrations of bioactive substances, the toxins, which result in large fish and bird kills as well as human illness. Over 43 species of dinoflagellates produce bioactive substances (including toxins) often present during the Red Tides, yet the teleologic purpose for the production of these bioactive substances is still unknown. Some scientists postulate that the toxins play an ancillary role in dinoflagellate metabolism, while others believe these substances are used as a competitive advantage by the dinoflagellate against other organisms (Baden 1983, ILO 1984, Carmichael et al, 1986, Halstead 1988, Winter et al, 1990, Miller 1991). The toxins produced by marine dinoflagellates include the paralytic shellfish poisons, the neurotoxic shellfish poisons, the diarrhetic shellfish poisons, and the ciguatera poisons (Baden 1983). The most recent addition to the toxic marine unicellular algae are the diatoms, and certain species produce the amnesic shellfish poisons (Wright et al. 1989).

By virtue of the ecological life-style of the incriminated toxic "microalgae", food webs

dependent upon filter-feeding as a strategy will tend to concentrate "red tide" toxins. Conversely, toxins from epibenthic forms tend to enter foods by initial "grazing" of herbivorous or omnivorous fishes, with subsequent bioconcentration by predation. Hence, toxic seafood sources will either be of fish or shellfish types, depending on where and how the toxic life form is first consumed.

In the past, these marine toxin illnesses have been predominantly confined to seafood-dependent populations, often located on islands or in coastal areas, each intoxication type occurring in relatively circumscribed areas of the world. However, with increased tourism and the international fishing trade, as well as the overall increased popularity and importance of shellfish and fish in human diets, marine toxin diseases are being increasingly encountered worldwide (Halstead 1988, Lange et al, 1992). In addition, it is hypothesized that human-generated environmental changes such as reef destruction and eutrophication, may be responsible for apparent increased reporting in cases of human disease as well as increased incidence of red tides reported worldwide (Bagnis 1984a, Ruff 1989, Viviani 1992). There also is a body of evidence to indicate that man-induced transportation of cysts of toxic marine dinoflagellates (the seeds) or the dinoflagellates themselves occurs in 'spat' (young bivalve shellfish sold commercially to global markets for aquaculture) and ship ballast water (international regulations are now changing to require ship ballast water purging in the open ocean prior to docking).

Until recently, the medical diagnosis of marine toxin diseases was restricted to a clinical diagnosis based on the clinical history combined with bioassays of contaminated fish or shellfish. This has hampered the accurate diagnosis, development of treatment and the elaboration of an exact epidemiology of the extent of marine toxin diseases in human populations. In addition, the treatment for these diseases has been predominantly symptomatic and supportive, local remedies notwithstanding.

Appropriately, the majority of work in the past has emphasized the development of surveillance methods and bioassays of contaminated materials to prevent human contact with contaminated shellfish and fish. In both humans and animals, marine toxin diseases have often occurred in disease clusters, such that follow-up of individual cases has led to the discovery and prevention of new cases (Dembert et al, 1981). However, with increasing prevalence worldwide, as well as increased scientific knowledge, new work is focusing on understanding the pharmacologic and pathologic mechanisms in humans in order to develop biomarkers for improved clinical diagnosis, prevention and epidemiology, and possibly improved treatment capabilities.

## **2. Molecular Toxicology of Marine Toxins**

### **A. Specific Receptor Site Interaction: Physiologic Biomarkers of Susceptibility**

The naturally-occurring marine toxins responsible for Paralytic Shellfish Poisoning, Neurotoxic Shellfish Poisoning, Amnesic Shellfish Poisoning, Diarrheic Shellfish Poisoning, Fugu Poisoning, and Ciguatera exert their effects in the nanomole to picomole per kg body weight ranges (Yasumoto and Murata 1993). By virtue of their highly specific and potent deleterious effects on living systems, specific receptor-ligands were postulated as the pharmacologically significant event in the onset of toxicity even before the chemical identity of the toxin(s) were known (see Baden, 1983 for a review). Of those toxins now known to the seafood safety regulators, four types are known to interact with specific orphan receptors located on nerve membrane glycoproteins, one type is a potent muscle enzyme inhibitor, and one interacts specifically with a central nervous system neurotransmitter receptor site. Each interacts in a highly specific ligand manner with its

biological receptor site and leads to a multitude of cascade processes by which the ultimate toxicological response is manifested (figure 1) (adapted from Cooper et al. 1982).

**FIGURE 1 HERE.**

It follows in a human and animal susceptibility sense that those individuals, groups, strains, or races who possess the receptor, enzyme, or binding site face the greatest peril. In an ancillary sense, compromised individuals who have impaired function in one of these areas might also be considered to be at increased risk.

It is in this regard that molecular toxicologists are making their current greatest contribution to seafood/marine toxin safety issues. Identification and characterization of the specific toxin (ligand)/receptor interactions will provide the raw information which leads to prediction of susceptibility, identification of bound biomarkers in intoxicated individuals, and ultimate development of specific therapeutic intervention for poisoning.

**B. Toxins as Ligands for Orphan Receptors**

Orphan receptors is a term used by toxicologists to define a specific binding site which exhibits a high affinity and avidity for exogenous xenobiotic ligands. That is to say, orphan receptors are located on essential endogenous macromolecules which bind environmental toxicants as a first step in expression of organism toxicity. The term "orphan" refers to the concept that, although the specific binding or receptor site may be well-defined, there is no known endogenous ligand which interacts with the site to induce the same biological response. Hence, this definition may be a fleeting one, for as soon as an endogenous ligand is identified, it is no longer an orphan receptor!

In any case, at this writing there are four different natural marine toxin types which interact with ligand receptor sites located on the voltage-sensitive sodium channel (VSSC).

**FIGURE 2 HERE.**

The VSSC is composed of three non-identical glycoprotein subunits, associated in a 1:1:1 stoichiometry: the  $\hat{\alpha}$ -subunit which is composed of four homologous domains and a total molecular weight of ca. 360,000; and the  $\hat{\alpha}_1$  and  $\hat{\alpha}_2$  subunits of molecular weight 36,000 and 33,000, respectively (Catterall, 1992). Acting in concert, these three subunits modulate the flow of sodium ions across excitable membranes by modifying allosterically in response to changes in membrane potential. That VSSC activation and inactivation involves a major conformational change which occurs in response to disruption of protein-membrane charge complexes is supported by the original work of Hodgkin and Huxley (1952 a-d), and by the description of "gating" currents which represent the actual movement of charges that accompanies activation (Armstrong, 1992). According to Catterall (1992), "In all probability these charged groups are amino acids in the protein structure which are altered by conformational modification".

Translated as a single polypeptide chain, the primary structure of the  $\hat{\alpha}$ -subunit reveals a four-fold homology, with 6 transmembrane helices labeled S1-S6 in each of the 4 domains (the extended structure in figure 2) (Noda *et al.*, 1984). When inserted into the membrane, the  $\hat{\alpha}$ -subunit arranges itself with the four homologous domains arranged in quadrants, as shown in the condensed illustration in figure 2 (viewed from the outside towards the interior of the membrane). Presumably, the "ion pore" itself is located in the center of the four domains.

Transmembrane helices S1,S2,and S3 are relatively negatively charged helices, S4 is highly positively charged (+++ in figure 2), and S5 and S6 are largely hydrophobic. The S4

transmembrane helix in each domain is particularly interesting in that every third residue is an arginine or other positively charged amino acid, the remaining residues in the series being hydrophobic [Noda *et al*, 1984]. When arranged in a helix, computer molecular modeling reveals - amino functions which extend from the helix much like the treads on a circular staircase. The S4 "sliding helix model" (Catterall 1986a) is proposed as an explanation and identity of the gating or voltage sensor of the channel. Changes in membrane potential disrupt the charge pairing between "+" charged  $\alpha$ -amino functions and "-" charged residues in the S1, S2, and S3 segments of the individual domains, leading to an outward twisting of the four S4's, ultimately exposing or opening the channel to sodium ion flow. S5 and S6 are thought to render the subunit's effective character as hydrophobic which aids insertion into the membrane (Catterall, 1986b).

On the channel, it is the highly ordered 3-dimensional topography of the  $\alpha$ -subunit which exhibits a profound affinity for saxitoxin and tetrodotoxin at Orphan Receptor Site 1 (A in figure 2) (Catterall *et al*. 1979), and for brevetoxins and ciguatoxin at Orphan Receptor Site 5 (B in figure 2) (Poli *et al*. 1986, Baden *et al*. 1989). Binding of these ligands leads to conformational changes in the "ion pore" component of the channel, much like membrane electrical field-induced modification. The spatial relationship of the voltage sensors of the channel to the toxin binding sites is a topic of current research activity, as are the uses of the toxins as molecular "probes" or "rulers"; measuring devices for determining topographic distances and conformational changes which activate or block the transmembrane. In other words, the toxins modulate the allostery of the transmembrane pore by merely binding to a specific orphan receptor site. Thus biochemistry and electrophysiology may find common ground in the study of toxin-induced channel modulation.

Site 1 Toxins: Saxitoxin (figure 3) and tetrodotoxin (figure 4) inhibit sodium ion flow when applied to nerves, muscle, and isolated channels (Narahashi, 1974). Their effects are

effected from the exterior of the pore, and molecular studies including sodium channel deletion mutations indicate that the specific binding site for the Site 1 toxins is located at point "A" in figure 2. This orphan receptor site is believed to be located near the extracellular side of the channel, an hypothesis supported by site directed mutagenesis (reduced binding in the absence of glutamate 387 at the extracellular loop between S5 and S6 of domain 1, just outside S6 (Noda *et al*, 1989), by the lack of activity of STX or TTX upon intracellular injection (Ritchie and Rogart, 1977), and by several lines of photoaffinity biochemistry and chemical modification of carboxyl residues.

### **FIGURES 3 AND 4 HERE.**

There is a pH dependence of binding (Henderson *et al*, 1973; Ritchie and Rogart, 1977), under optimum conditions each toxin binds with a dissociation constant of about 1 nMolar, and in a 1:1 stoichiometry with the subunit (Kreuger *et al.*, 1979; Lawrence and Catterall, 1981). The guanidinium character of the two toxins is considered to be essential for their activity, the guanidinium being viewed by the channel as transportable, but the rest of the molecule is prevented from transversing the channel based on ion charge-pairing. Hence, these two toxins act as plugs (Kao, 1993) or lids on the channel. It is generally agreed that at least three of the four S6 transmembrane helices act in concert to form the Site 1 binding site at or near the extracellular side of the pore and create the classical "3-point site" of attachment of ligand to active site. Thus, the site 1 toxins will aid in the complete description of the topography surrounding sodium channel  $\alpha$ -subunit exterior pore dimensions.

Site 5 Toxins: Both brevetoxin types (figure 5) and ciguatoxin (figure 6) interact with the polyether ladder toxin binding site known as Site 5. Binding at this site causes channels to open at normal resting potential, slows normal inactivation of channels, and often results in repetitive firing in nerves. Like TTX and STX, a specific binding site has been described (Poli, 1986; Trainer et al, 1991) which exhibits a 1:1 stoichiometry with channels, and which exhibits half-maximal binding

in the nanomolar concentration range. Specific labeling of Site 5 with brevetoxin photaffinity probes on the rigid G-H-I-J ring side chain of PbTx-3 (figure 5 R1) revealed a domain 4-specific high affinity site, located between S5 and S6 (B in figure 2) and its specific localization may exist in the portion of the extracellular loop which lines the interior surface of the pore itself (Trainer et al, 1991). Thus, the initial binding event in the toxicity of brevetoxins is likely binding of this rigid region to the receptor site.

### **FIGURES 5 AND 6 HERE.**

Considerable molecular modeling and structure/activity work has been carried out with brevetoxin PbTx-3 (a PbTx-2-type toxin). All the polyether ladder toxin possess considerable flexibility across their lengths, contain similar rigid structural features topographically similar to the G-H-I-J ring region of PbTx-2, and carry a lactone or extant lactone electrophile on the end distal to the rigid portion. The lactone appears to be essential for activity; at least an electrophile is required (Baden et al., 1993). The overall lengths of the molecules range from 26 Å to 35 Å, and the proposed S5-S6 binding site extends approximately 6 Å into the membrane. The present conclusion is that these toxins exert their effects near the inside of the membrane, and have the potential to affect both activation and inactivation (Trainer et al, 1993).

The model for the activation properties of polyether toxin involves a disruption of the charge-charge pairing of the "+" charged S4 helices with the S1, S2, and S3 helices by the toxin lactone electrophile substituting for one of the S1, S2, or S3 "-" charges (Rein et al, 1993 a). Just as electrical stimulation disrupts the charge-pairing, so does toxin intercalation into the ion-pairing. An interesting observation (Jeglitsch et al, 1993) involves the application of brevetoxin under patch clamp conditions to reveal 5 separate sub-conductance states. A possible explanation for the multiplicity of subconductance states induced by the toxin is an allosteric one, in which the four individual domains must sequentially "flip" into a favorable conformation for channel activation

(figure 7). Were brevetoxins to have an ion-pair disrupting effect in each of the domains (while only binding to domain 4), five subconductance states would be revealed (one normal and one subconductance state for each of the four S4 regions). Thus, these toxins may possess the ability to assist in description of which charge-charge complexes represent the first, second, and so forth in the ordered sequence of subunit allosteric modulation for channel activation.

**FIGURE 7 HERE.**

For several years, the effect of brevetoxins and ciguatoxin on inactivation has been debated. Slowed inactivation is most assuredly seen in invertebrate systems (Huang et al, 1984), a result thought by some to be an artifact of the model system. Patch clamping of rat endothelial neurons has illustrated in this mammalian system that indeed brevetoxin alter the kinetics of inactivation (Jeglitsch et al. 1993).

Based on experimental evidence (Huang et al. 1984, Jeglitsch and Schreiber 1991, Jeglitsch et al. 1993), on the molecular mechanism and primary structure of the "inactivation particle" (D in figure 2) (Catterall 1992), and on molecular modeling studies (Rein et al., 1993a,b; Yasumoto and Murata, 1993) brevetoxins may indeed affect inactivation by a similar disruption of normal ion-pairing during the inactivation process.

C. Toxins as Enzyme Inhibitors

The toxins responsible for Diarrhetic Shellfish Poisoning world-wide are based on the structure of okadaic acid (figure 8). The toxin was first isolated from a sponge (Tachibana *et al*, 1981) and later described as having its biogenesis in a commensal dinoflagellate and as a principal causative organism in outbreaks of DSP (Murikami *et al*, 1982). There are now described numerous derivatives of okadaic acid, some of which occur in the various dinoflagellate species

which have now been incriminated as well as those found in shellfish transvectors of toxin from seawater to man. The R1 functionality, a proton in okadaic acid, is a common site of derivatization and several o-acyl fatty acid derivatives have been isolated and characterized (Murata *et al*, 1982). The latter derivatives appear to predominate in shellfish that concentrate the toxins. The R2 position is either a hydrogen (in OA) or a methyl group and these are known as the dinophysis toxins (Yasumoto and Murata, 1993). Two other toxins, yessotoxin and the pectenotoxins, co-occur with okadaic acid derivatives but will not be discussed here due to the lack of information on human incidence of disease.

**FIGURE 8 HERE.**

Unlike the toxins described earlier that exhibit intraperitoneal animal lethality in the fractions-of-micrograms per kilogram body weight range, the DSP toxins more approximate 200-500 g/kg body weight. The most profound effects of okadaic acid appear to be smooth muscle effects, which in 1982 were traced to modification of protein phosphorylation (Shibata *et al*, 1982). This is reflected in the prolonged contraction of smooth muscle in arteries (Shibata *et al*, 1982) and is due to an inhibition of myosin light chain phosphatase (Takai *et al.*, 1987). Cohen and others have since shown that OA and its derivatives are potent inhibitors of protein phosphatase 1 (PP1) and protein phosphatase 2a (PP2A) (Cohen, 1989; Haystead *et al*, 1989). These two enzymes are essentially ubiquitous in mammalian cells, and serve numerous regulatory roles in enzyme activation and inactivation, in cascade systems, transport across cell membranes, and in intermediary metabolism. Thus, an exogenous compound which can so systematically and completely deregulate more than 20 phosphoproteins would be expected to play havoc in public health circles. In fact, there is also considerable literature which points to OA as a potent non-TPA type tumor promoter, again a finding not unexpected owing to its disruption of vital cellular regulation (Fujuki *et al*, 1988).

The exact mechanism by which okadaic acid inhibits protein phosphatases is thought to relate to the potential role of the toxin for the dinoflagellate itself; that of regulating protein phosphorylation. In fact, there is some mounting evidence that okadaic acid is in fact a phosphatase regulator in *Prorocentrum* species which assist the cells in scavenging organic phosphate (Kinoshita, personal communication). There is much sequence homology in the catalytic subunits of protein phosphatases (Bernt *et al* 1987), and so it is not surprising to see effects on both PP1 and PP2a. However, the inhibition is not at the substrate binding site for okadaic acid inhibition exhibits non-competitive or mixed inhibition patterns with respect to substrate.

It is possible however, that the PP inhibitor exerts its activity via the regulatory subunit binding site on PP1 and PP2a. To decide if this is the case, competition studies employing both the regulatory subunit (normally a peptide in mammalian systems) of the PP1 or PP2a, and okadaic acid. In all likelihood, competitive binding at the regulatory subunit binding site will be observed. The reason then for its exquisite selectivity for PP1 and PP2a is that okadaic acid is a regulator of protein phosphatases in some systems naturally, and when it comes in contact with mammalian protein phosphatases it seeks to regulate and inactivate the phosphatase by combining with a regulatory subunit binding site.

Exactly how okadaic acid binds, and what the molecular topography of the molecule must be for potent inhibitory activity is not known with certainty. However, there is currently much interest in a similar type of PP1 and PP2a inhibition activity exhibited by the cyclic microcystin peptides, and molecular modeling of okadaic acid reveals some cyclized forms which appear as predominant conformers. The future of okadaic acid research may provide clues to exactly how protein phosphatases are regulated in mammals, and it may provide some interesting information on how lower life forms have evolved homologous mechanisms for regulating the phosphorylation and

dephosphorylation of their cellular proteins.

#### D. Toxins as Neurotransmitter Agonists

The final (known!) toxin is domoic acid (figure 9), an excitatory dicarboxylic amino acid similar to kainic acid (Baden and Trainer, 1993). Like kainic acid, and glutamic acid the normal neurotransmitter, domoic acid competes for glutamate receptors in the CNS. Application of, or exposure to, domoic acid leads to persistent and debilitating necrosis of the hippocampal region due to high affinity and avidity for kainate receptors. All of the effects of domoic acid mimic kainic acid. Kainate and domoic acid are purely competitive in binding activity; the latter exhibits a 3-5 fold greater affinity over the former (Angst and Williams, 1987), and in some receptor systems *in vivo* domoic acid exhibited a 20-fold greater potency than did kainate (Debonnel *et al*, 1989).

#### **FIGURE 9 HERE.**

Domoic acid has been recognized as a human public health threat only since 1987, a there is still a great deal to learn about its molecular mechanism. That this toxin binds to the kainate receptor subtype of glutamate receptor is reassuring in a research sense for a great deal is known about the kainate receptor in CNS. Rapid progress in complete characterization can be expected in this area.

### **3. Epidemiology, Diagnosis, and Management of Marine Toxins Exposure**

#### A. Paralytic Shellfish Poisons (PSP)

##### 1. background & epidemiology:

(Southcott 1979, Baden 1983, Shimizu 1984, Halstead & Schantz 1984, ILO 1984, Carmichael et al, 1986, Saunders 1987, Halstead 1988,

Viviani 1992, Kao 1993)

PSP is a marine toxin disease with both gastrointestinal and neurologic symptoms reported worldwide. It is caused predominantly by the consumption of contaminated shellfish.

PSP was first recognized by Vancouver (1801) in the Pacific northwest of North America in 1793 with the report of one death. Further outbreaks were reported as food poisoning from 18th century from Europe, North America, Japan, South Africa and New Zealand. Red tides were even reported in the ancient Mediterranean in the writings of Homer (Iliad), Tacitus and early European navigators (Bower et al, 1981, Halstead 1988). In the past decade, reports of red tides and PSP cases have occurred with increasing frequency throughout the world not only in endemic areas but also from importing countries, including from Malaysia, Solomon Islands, Tasmania, Philippines, Thailand, Brunei Darussalam, South America, as well as the European Atlantic coast (Hermes & Viloso 1983, Mee et al, 1986, Eason & Harding 1987, Rodrigue et al, 1990, Saldate Castaneda et al, 1991, Viviani 1992). Worldwide, about 1600 cases of PSP are estimated to occur each year (Halstead 1984); over 24 deaths occurred out of the 905 cases reported cases from 1969-1983 (ILO 1984). It is not clear if this apparent increase in the incidence of PSP is due to increased reporting or to an actual increase in incidence. However, Kao (1993) believes that the clinical manifestations of PSP are so characteristic that this increase in incidence is real.

Dinoflagellates (mainly the species in genus *Gonyaulax*, recently reclassified to *Alexandrium*) are the source of the PSP marine toxins. These unicellular dinoflagellates develop algal blooms throughout the world for unknown reasons, although a variety of factors have been studied, including change in weather, upwellings, temperature, turbulence, salinity, and transparency. In addition, new theories are examining the importance of hyper-trophication of terrestrial origin in the development of toxic algal blooms; for example, possibly due to aquaculture pollution, there was an emergence of red tides in Faroe Islands in 1984 (Viviani 1992). Of note, these red tides can be toxic or not, again for unknown reasons. Significant epidemics of PSP can occur in humans in the absence of a known red tide (Rodrigue et al. 1990).

These dinoflagellates produce at least 12 toxins which are tetrahydropurines, and heat and acid stable. Shellfish, once having accumulated the toxins, metabolize them to as many as six additional potent materials, and possess the enzymology to interconvert toxins from one derivative to another. Saxitoxin was the first characterized and the best understood; there are at least 12 others (including neosaxitoxin, gonyautoxin I, gonyautoxin III, decarbamoyl saxitoxin; less toxic are gonyautoxin II, IV, V-VIII, and sulfocarbamoyl gonyautoxin I & IV)) which have been divided into three groups predominantly based on chemical substitutions: carbamate, N-sulfocarbamoyl and decarbamoyl components. In general, they have similar chemical properties to saxitoxin. However, this multiplicity of toxins associated with PSP makes accurate detection and quantification difficult. Although PSP is an illness which occurs worldwide, there is complete heterogeneity in terms of toxin composition and saxitoxin is not always the major constituent (Baden 1983).

The major transvector for PSP are the bivalve molluscs (mussels, clams, oysters, with the Alaskan butterclam having the highest concentrations) (Sommer & Meyer 1937). PSP toxins are also found in certain crabs and snails which feed on coral reef seaweed. The transvectors accumulate the toxins via feeding in their digestive organs and soft tissues, apparently without harm to the transvectors.

Humans, birds and fish can all be affected by PSP toxins. Herbivorous zooplankton is the primary transvector which can in turn transmit the toxin to fish and possibly other marine creatures which consume zooplankton (Baden 1983). The usual route for humans is the consumption of raw or cooked contaminated shellfish.

There has been only one case of human contamination through consumption of contaminated fish and bird kills in Indonesia. In this case, the whole fish was consumed including the viscera which could be contaminated with PSP from shellfish consumption by the fish prior to death (MacLean & White 1989, Viviani 1992).

The overall mortality is reported to be 1-10% for people with clinical PSP, with death 1 to 12 hours post ingestion; the mortality rate does depend on the availability of emergency medical care. In a PSP outbreak on the Pacific coast of Guatemala in July-August 1987 with 187 people, there were 26 fatalities (14%), but the case fatality was highest in young children (50%)

(Rodrigue et al, 1990).

## 2. neural mechanism:

Saxitoxin is the most well known of the PSP associated toxins. It is a heat stable neurotoxin. In mice, the saxitoxin LD50 parentally is 3-10 ug/kg body weight and orally is 263 ug/kg body weight (death within minutes of respiratory failure). Humans are the most sensitive to saxitoxin; the oral dose in humans for death is 1 to 4 mg (5,000 to 20,000 mouse units) depending upon the age and physical condition of the patient (see below) (Wiberg & Stephenson 1960, Shimizu 1984, Winter et al,1990). It is rapidly absorbed through the gastro-intestinal tract and excreted in the urine.

Experimental animal work has shown that saxitoxin and its analogues cause predominantly peripheral neurologic intoxication, unless directly injected into the CNS, especially with respect to respiratory paralysis (Borison et al, 1980a, Borison et al, 1980b, Baden 1983, Winter et al, 1990). Saxitoxin inhibits the temporary permeability of Na<sup>+</sup> ions by binding tightly to a receptor site on the outside surface of the membrane very close to the external orifice of the sodium channel. In fact, neurophysiologic studies using saxitoxin as a probe helped to show that Na<sup>+</sup> and K<sup>+</sup> act independently with separate membrane channels. It is a blocking agent that reduces the number of conducting Na<sup>+</sup> channels by occupying some site near the outer opening in a 1:1 high affinity specific receptor binding. This prevents sodium ions from passing through the membranes of nerve cells, thus interfering with the transmission of signals along the nerves. The resulting widespread blockade prevents impulse-generation in peripheral nerves and skeletal muscles (Murtha 1960, Evans 1967, Schwartz 1971, Narahashi et al, 1972, Adams et al, 1976, Bower et al, 1981, Kao 1983, Winter et al, 1990, Viviani 1992).

Saxitoxin has a direct effect on skeletal muscle by blocking the muscle action potential without depolarizing cells; it abolishes peripheral nerve conduction but with no curare-like action at the neuromuscular junction. Direct cardiac effects are minimal and transient, therefore there is little or no hypotension (Murtha 1960, Evans 1967, Schwartz 1971, Narahashi et al, 1972,

Adams et al, 1976, Bower et al, 1981, Kao 1983, Winter et al, 1990, Viviani 1992).

Saxitoxin and tetrodotoxin (the etiologic agent of Pufferfish Poisoning; see below) are very similar in terms of their clinical effects and they cannot be distinguished easily by traditional mouse bioassay. Saxitoxin and tetrodotoxin both block or close the passage of sodium ions through the channel. Saxitoxin binds at a sodium channel receptor reversibly and in a concentration dependent fashion; it is specific even at low concentrations ( $10^{-9}$  M). When saxitoxin is mixed in small amounts with many classes of anaesthetics, the anesthetic action is greatly extended in a multiplicative fashion; this allows for decreased doses of anaesthetic for the same effect. Saxitoxin and tetrodotoxin have both been important in the characterization of sodium channels in myelinated and unmyelinated nerve membranes, and in the study of related diseases such as MS; both block the inward current of sodium ions at equally low concentrations of  $10^{-7}$  to  $10^{-9}$  M and occupy the same receptor sites at the sodium channel although with different chemical structures (Shimizu 1984, Schantz & Johnson 1992).

### 3. clinical presentation

Ingestion of molluscs contaminated with PSP results in the following clinical picture (Bower et al, 1981, Kao 1993). Five to 30 minutes from consumption, there is slight perioral tingling progressing to numbness which spreads to face and neck to moderate cases. In severe cases, these symptoms spread to the extremities with incoordination and respiratory difficulty. There are medullary disturbances in severe cases evidenced by difficulty swallowing, sense of throat constriction, speech incoherence or complete loss of speech, as well as brain stem dysfunction. Within 2-12 hours, in very severe cases, there is complete paralysis and death from respiratory failure in absence of ventilatory support. After 12 hours, regardless of severity, victims start to recover gradually and are without any residual symptoms within a few days (Bower et al, 1981, ILO 1984, Halstead 1988).

In one patient with acute bulbar and respiratory paralysis following ingestion of saxitoxin-contaminated clams, serial electrophysiologic observation showed prolonged distal motor and sensory latencies, slowed conduction velocities, and moderately

diminished amplitudes at the outset. There was a return to normal over 5 days. This illustrates the incomplete reversible sodium channel blockade caused by saxitoxin and the other PSP toxins (Long et al, 1990).

Other symptoms include headache, dizziness, nausea, vomiting, rapid pain, and anuria. There is no loss of consciousness and the reflexes are unaltered except maybe pupillary size and sight may be temporarily lost. As opposed to tetrodotoxin poisoning, there is rarely significant hypotension. Symptomatology is essentially identical for Pacific and Atlantic cases, although gastrointestinal symptoms may be more prominent in the Atlantic (ILO 1984, Halstead 1988).

Lactic acidosis of unexplained origin has been found in experimental animals administered saxitoxin (Franz & LeClaire 1989). Because the toxin interferes with respiratory function, this acidosis could not be compensated for naturally by hyperventilation. It is possible that this lactic acidosis has also been seen in human cases of PSP (Kao 1993).

In an outbreak of PSP in southern Taiwan, 3 of 5 patients had significant increase of creatine kinase MB level without correlation with the severity of poisoning; this abnormality resolved completely with recovery (Cheng et al, 1991). There are no reports of chronic disease associated even with severe cases of PSP except for some residual headaches, memory loss and fatigue for up to 2 weeks reported in a recent epidemic in Guatemala (Rodrigue et al, 1990).

The overall mortality (case fatality rate) was about 8.5%-9.5% in two large series (Meyer 1953, Ayres and Cullum 1978). However, as has been mentioned, the Guatemalan 1987 outbreak on Pacific coast had a case fatality rate of 14% which was even higher in young children (50%). It is possible that children may be more sensitive to PSP toxins than adults (Rodrigue et al. 1990). In addition, the access to emergency medical services in acute cases is crucial to the prognosis.

The differential diagnosis of this clinical scenario of an acute gastrointestinal illness with recent shellfish ingestion would be bacterial or viral gastroenteritis. The neurologic manifestations are more consistent with poisoning by other marine toxins such as NSP and pufferfish poisoning, or even recent organophosphate pesticide poisoning.

#### 4. diagnosis

The clinical scenario is the primary method of diagnosis initially. Recent shellfish ingestion, often but not always associated with known red tide, and an acute gastrointestinal illness with neurologic symptoms are part of the classic presentation. It is imperative to obtain samples of contaminated tissues and their source.

As mentioned above, each PSP epidemic is associated with different mixtures of the PSP toxins; this complicates the laboratory analysis of contaminated tissues. The mouse bioassay (time to death) of food extract is the recommended diagnostic method, (Sommer & Meyer 1937, Association of Official Analytical Chemists 1980) but it cannot distinguish between tetrodotoxin and other PSP toxins. The oral dose in humans for death is 1 to 4 mg (5,000 to 20,000 mouse units) depending upon the age and physical condition of the patient (see below). A mouse unit [MU] is defined as the minimum amount needed to cause the death of an 18 to 22 g white mouse in 15 minutes (Wiberg & Stephenson 1960, Shimizu 1984, Winter et al, 1990).

Radioimmunoassay and indirect enzyme-linked immunoabsorbent assay (ELISA) have been developed for saxitoxin but not all PSP toxins (Carlson et al, 1984). HPLC analysis method for all the PSP toxins has been developed with good correlation with mouse bioassay in terms of quantification (Sullivan et al, 1983, Halstead 1988).

#### 5. management & treatment

In general, supportive measures are the basis of treatment for PSP, especially ventilatory support in severe cases. In animals, artificial respiration is the most effective treatment. Without supportive treatment, upto 75% of severely affected persons die within 12 hours. Use of anticholinesterase agents are not recommended, and could actually be harmful (Murtha 1960, ILO 1984, Halstead 1988, Brown & Shepherd 1992, Kao 1993).

When the ingestion of contaminated food is recent, gut decontamination by the gastric lavage and administration of activated charcoal or dilute bicarbonate solution is recommended. Care must be taken concerning aspiration with the neurologically compromised patient. Anticholinergic drugs were ineffective, while DL amphetamine (benzedrine) was most effective in aiding the artificial respiration and decreasing the recovery period. Use of anticholinesterase agents are not recommended, and could actually be harmful (Murtha 1960, Bower et al, 1981, ILO 1984, Halstead 1988, Brown & Shepherd 1992, Kao 1993).

The lactic acidosis of unknown origin seen in experimental animals and possibly humans can be treated by assisted ventilation, fluid therapy and periodic monitoring of the blood pH. It is possibly that the fluid therapy will also assist in the renal excretion of toxin (Kao 1993).

Many endemic areas have traditionally used local treatments with variable success. In the Philippines, a drink of coconut and brown sugar is administered; demonstrations in mice show that these ingredients may have active detoxification substances (Viviani 1992).

Recent work by Kaufman et al (1991) has focused on the development of a therapeutic antiserum, although this is complicated by the wide range of PSP toxins. In a rabbit, a tetrodotoxin-formaldehyde-keyhole limpet hemocyanin conjugate was used to immunize a rabbit for the production of anti-toxin antiserum which cross-reacted against tetrodotoxin and saxitoxin. In a quantitative in vitro assay, it was able to protect cells in a dose dependent manner from the effects of either toxin, and the antiserum was able to passively protect mice challenged in vivo with either toxin. Finally, hybridomas producing monoclonal antibodies against toxin were obtained from the spleens of mice immunized with the same conjugate (Kaufman et al, 1991, Viviani 1992).

As with many of the marine toxin induced diseases, the initial or index case(s) are often the tip of the iceberg. Therefore any cases of PSP should be reported to the appropriate public health authorities for follow up to ascertain other cases and to prevent further spread. And every effort should be made to obtain contaminated materials and their source.

Obviously the most effective form of PSP prevention is to eliminate human contact with contaminated shellfish and other transvectors. Surveillance and closures of commercial shellfish beds by monitoring the amount of PSP using the mouse assay are common practice throughout the world. For example, in the USA, PSP levels in edible shellfish greater than 800 ug PSP/kg by mouse assay means that commercial beds will be closed until they are monitored below this level; this action level is more than 10 times lower than the lowest level associated with human outbreaks [Anon 1965, ILO 1984]. Furthermore, there is active monitoring of algal blooms with fish and bird kills.

Ozonation can remove low levels of toxins from soft-shell clams but not if the clams have retained toxin for long periods of time; some industrial canning processes may lead to a decrease in PSP concentration (Halstead 1988, Viviani 1992). Biological controls such as using parasitic dinoflagellates to attack the red tide (for example *Amoebophrya ceratii* parasitizes a variety of dinoflagellates responsible for PSP) have been considered (Viviani 1992).

## B. Tetrodotoxin (Pufferfish Poison) (Fugu)

### 1. background & epidemiology

Pufferfish Poisoning or tetrodotoxin intoxication (Fugu) is a gastroenteritis with severe neurologic manifestations similar to PSP (Hashimoto 1979, Southcott 1979, ILO 1984, Halstead 1988). It is found worldwide and is associated predominantly with the ingestion of pufferfish. Fugu poisoning was known as a fatal disease by the ancient Chinese as early as 2800 BC (Kao 1966). An Egyptian carving of 2700 BC warned about eating fish without scales, probably referring to the pufferfish family.

Fugu poisoning is not due to dinoflagellates. The associated toxin, tetrodotoxin, is found in the order of fish known as Tetraodontiformes especially in the family Tetraodontidae (pufferfish). Over the years, substantial evidence has been compiled to

implicate marine bacteria in the production of toxic puffers (Kodama 1985, Kodama 1988, Kodama 1993). These bacteria colonize the gut and skin mucosal layers of the puffer following infection, and produce persistent levels of tetrodotoxin which the fish sequesters in gonads, liver, and to some extent muscle.

The tetraodontiformes are found in both tropical and temperate waters with several hundreds of species (Vietmeyer and Scherschel 1984). Concentrations of tetrodotoxin vary with season (especially high in Japan during October through March) and are especially high in the ovaries, livers and intestine; they are rarely high in muscle except for *Lagocephalus lunaris lunaris*. Other marine organisms are associated with tetrodotoxin: the Japanese ivory shell and the trumpet shell. Tetrodotoxin is also found in the skin of certain frogs and it is the principal poison in venom of blue-ringed octopus.

The most toxic pufferfish are found along the coasts of China & Japan where their consumption is considered a delicacy (known as "fugu" in Japan), and where they are eaten only after preparation by specially trained chefs (Vietmeyer 1984). The majority of cases of Fugu reported are due to this intentional ingestion of pufferfish both in Japan and other locations in the Far East (Lyn 1985, Kan et al, 1987). In Japan 1974-1979, 60 cases were reported with 20 deaths (Kainuma 1981, ILO 1984). About 50% of fatal food poisonings in Japan each year are due to eating fugu with a death rate in these reports of upto 59.5% reported cases (Halstead 1967). Of note, in Japan, cases are more common among men than women, probably related to eating habits (Halstead 1988). There are also exported cases reported in Europe due to mislabeling (Pocchiari 1977) and elsewhere in the world due to ignorance (Joy-Sobrino et al, 1985). Thus, Fugu is a very circumscribed public health problem with a high mortality.

Of interest, intentional use of tetrodotoxin as a sublethal poison has been anecdotally implicated in the "Zombi Phenomenon" from Haiti and other parts of the Caribbean, although this has been disputed (Davis 1985, Anderson et al, 1988, Davis 1989). There are cases reported from Japan of complete recovery in victims presumed dead for up to 7 days (Halstead 1988).

## 2. neural mechanism

Tetrodotoxin is one of the most toxic of the natural toxins (Kao 1972). LD50 in cat is less than 10 ug/kg and lethal ingested dose for humans is 5-30 mg/kg wet tissue. (Vietmeyer & Scherschel 1984) It is classified as an aminoperhydroquanzole with molecular weight of 319 (Schantz & Johnson 1992).

The primary effect of the tetrodotoxin is to block the excitability of nerve axons by reducing their permeability to the inward flow of sodium ions. During the action potential, the membranes of excitable cells undergo a transient increase in sodium permeability; the toxin acts by preventing this flow of sodium ions by blocking the channels through which the sodium ions flow. In addition, the sodium pump inhibition prevents the usual increase in permeability to sodium ion, but leaves the potassium ion permeability unaltered. It blocks the sodium channels in a 1:1 fashion. Tetrodotoxin has been one of the most widely used tools for selective blockade of sodium channels in neurophysiology (Kao 1966, Narahashi et al, 1964, Fuhrman 1967).

Tetrodotoxin affects the emetic chemoceptor triggerzone of the area postrema. It causes severe hypotension at doses as low as 0.5 ug/kg (as opposed to saxitoxin) due to the progressive paralysis of blood vessels with resultant decrease in peripheral vascular resistance (Kao 1972). It acts on conduction in both somatic and sensory nerves. Tetrodotoxin acts on sympathetic nerve fibers, and on medullary centres. It reduces skeletal muscle excitability and decreases the contractile force of heart. Tetrodotoxin particularly affects the preganglionic cholinergic and somatic motor nerves. The respiratory failure associated with tetrodotoxin intoxication is due to the disappearance of the action potential in diaphragm before the phrenic nerve. Tetrodotoxin may have central nervous system effects (ILO 1984).

Compared with saxitoxin, Tetrodotoxin is slightly less neuroactive but has more prolonged effects. It is less easily reversed and creates small transient potentiation of maximal muscular contraction with subliminal doses. Tetrodotoxin has less effect on muscle fiber (saxitoxin can cause neuromuscular muscle weakness without hypotension), however it causes hypotension via effect on the vasomotor tone through preganglionic fibers or direct action on cardiac muscle. Finally, with tetrodotoxin, the antagonistic action of anticurare drugs such as edrophonium are stronger (Kao 1972, Bower et al, 1981, ILO 1984).

### 3. clinical presentation

The clinical presentation of Fugu is similar to PSP except for marked hypotension and a different ingestion history (Kao 1966, Hashimoto 1979, Bower et al, 1981, Spencer et al, 1987). There is a rapid onset of symptoms (5-30 minutes). Victims of PSP report weakness and dizziness, paresthesias in face spreading to the extremities, nausea, rarely vomiting, diarrhea, pallor, and sweating. In addition, diminished or absent superficial and deep reflexes have been reported as well as constriction then later dilation of the pupil. Progressive bulbar paralysis, extraocular muscle paralysis, and general body flaccid paralysis are seen with severe cases, although consciousness is usually maintained throughout. With severe cases there is gradual onset of respiratory distress with death within 6 hours of respiratory failure, and convulsions can occur upto 24 hours.

Fukuda and Tani have outlined 4 degrees of increasing tetrodotoxin intoxication by stages of progression (Halstead 1988):

- 1: oral paraesthesias, sometimes with gastroenteric symptoms
- 2: advanced paraesthesias, motor paralysis of extremities, intact reflexes
- 3: gross muscular incoordination, aphonia, dysphagia, respiratory distress, cyanosis, drop in blood pressure, but victim is conscious
- 4: Mental faculties impaired, respiratory paralysis, extreme drop in blood pressure with continued pulsation of heart for short time

The more rapid the onset of symptoms (ie. within 30 minutes) and the greater the degree of symptoms, the poorer the prognosis. If the person can survive 18-24 hours, the prognosis is favorable for complete recovery. No chronic manifestations of Fugu have been reported (Kao 1966, ILO 1984, Halstead 1988).

Although severe and prolonged arterial hypotension is usually reported as pathneumonic of tetrodotoxin poisoning, Deng et al (1991) reported cases with hypertension in 8/30 people in cluster in Taiwan after eating ovaries of unidentified fish with a positive mouse bioassay. 7/8 had signs of pre-existing mild hypertension and 1 individual died with pre-existing severe coronary artery disease. It would appear therefore that hypertension may be more common in those with pre-existing hypertension or coronary artery disease (Deng et al, 1991).

In a patient with severe tetrodotoxin intoxication serial nerve conduction studies were performed. Conduction velocities and amplitudes of muscle and sensory nerve action potentials were equally affected. There was neither temporal dispersion nor focal conduction block and the proximal F wave motor latencies were prolonged. There was rapid improvement with clinical recovery and with a parallel decrease in urinary excretion of tetrodotoxin over 4 days. The authors concluded that tetrodotoxin equally and reversibly affects myelinated nerve fibers throughout the entire length of the axon by lowering the conductance of sodium currents at the nodes of Ranvier. And they recommended the use of nerve conduction studies as a form of clinical Fugu assay as objective measure to monitor clinical Fugu toxicity (Oda et al, 1989).

#### 4. diagnosis

The mouse assay for PSP can be used. Tetrodotoxin can be distinguished from saxitoxin by bioassay before and after heating to 100 C at pH 1 for 25 minutes: tetrodotoxin loses most of its toxicity, not saxitoxin (Bower et al, 1981). Fluorescence spectrometry can also distinguish between PSP toxins (ILO 1984).

#### 5. management & treatment

The present treatment for Fugu is supportive and symptomatic. The major concerns are the respiratory failure and severe hypotension. When the ingestion of contaminated food is recent, gut decontamination by the gastric lavage and administration of activated charcoal or dilute bicarbonate solution is recommended. Care must be taken concerning aspiration

with the neurologically compromised patient. Hypotension may require infusion of fluids and/or catecholamines (Chew et al, 1983).

Clinical management for severe poisoning should include tracheal intubation for bulbar palsy and mechanic ventilation for respiratory insufficiency. It should be remembered that dilated unreactive pupils alone are not necessary consistent with brainstem neuromuscular block (Halstead 1988).

Anticholinesterase treatment has been used reportedly successfully in a few human cases, (Torda et al, 1973, Chew et al, 1983, Lyn 1985) using a slow iv injection of edrophonium after intubation and artificial respiration. However, this has not been substantiated experimentally. Golin & Larson (1969) in animals, found strychnine or pralidoxime combined with atropine effective with experimental animals; atropine by itself was found to increase the lethality in animals (Golin & Larson 1969). Kao (1966) found different results in animals. Kao found that tetrodotoxin blockade is not antagonized by either neostigmine nor edrophonium, and that the neuromuscular transmission is interrupted not at the endplates but at the motor axons and muscle membrane. With complete tetrodotoxin block, no neural stimulation was effective, nor could acetylcholine depolarization of the endplate lead to excitation of the muscle membrane. Tibbals (1988) found that neostigmine did not alter muscle paralysis when administered during established complete block with very severe cases. Therefore anticholinesterase treatment may depend on the degree of tetrodotoxin intoxication, and should only be used conjointly with artificial respiration and other supportive interventions (Tibbals 1988, Brown and Shephard 1992).

Recent work by Kaufman et al (1991) has focused on the development of a therapeutic antiserum, although this is complicated by the wide range of PSP toxins. In a rabbit, a tetrodotoxin-formaldehyde-keyhole limpet hemocyanin conjugate was used to immunize a rabbit for the production of anti-toxin antiserum which cross-reacted against tetrodotoxin and saxitoxin. In a quantitative in vitro assay, it was able to protect cells in a dose dependent manner from the effects of either toxin, and the antiserum was able to passively protect mice challenged in vivo with either toxin. Finally, hybridomas producing monoclonal antibodies against toxin were obtained from the spleens of mice immunized with the same conjugate (Kaufman et al, 1991, Viviani 1992).

As with many of the marine toxin induced diseases, the initial or index case(s) are often the tip of the iceberg. Therefore any suspected cases of Fugu should be reported to the appropriate public health authorities for follow up to ascertain other cases and to prevent further spread. And every effort should be made to obtain contaminated materials and their source. Obviously major prevention consists of not eating pufferfish! Or if you must partake of this Oriental delicacy, it is recommended that one employ the talents of a trained and certified Fugu cook (Hashimoto 1979).

### C. Neurotoxic Shellfish Poisons (NSP)

#### 1. background & epidemiology

NSP is a marine toxin-induced illness which presents as a milder gastroenteritis with neurologic symptoms compared with PSP; it is reported in relatively few geographic locations (ILO 1984, Halstead 1988, Viviani 1992). It is caused by the consumption of shellfish. In addition, inhalation of the NSP toxins can lead to a reversible upper respiratory syndrome.

NSP was first recorded by Walker in 1844 on west coast of Florida. The associated red tides are characterized by patches of discolored water, dead or dying fish and respiratory irritants in the air. Since then, NSP has been reported from the Gulf of Mexico, the east coast of Florida, the North Carolina coast, northern Spain, and eastern Mediterranean and Japan (Baden 1983, Viviani 1992). The most recent cases of suspect NSP come from New Zealand in 1993, where over a period of several weeks, approximately 135 cases were reported [France conf].

The causative organism, dinoflagellate *Ptychodiscus brevis* (= *Gymnodinium breve*), is restricted to the Gulf of Mexico and the Caribbean sea; a similar species resembling *P. brevis* occurs in Spain (Baden 1983). It is found especially during red tides in late summer and autumn months every 3-4 years on the west coast of Florida with massive fish and bird kills; fish kills associated with

these red tides have been estimated upto 100 tons of fishes per day (Halstead 1981). Of note, these red tides in Florida did occur even prior to significant pollution from human population; from 1844 to 1971, 24 reported fish kills were associated with red tides (Viviani 1992).

*P. brevis* produces 2 types of lipid soluble toxins: hemolytic and neurotoxic. The major toxin produced is brevetoxin B; there are also lesser amounts of Brevetoxin A, GB 3 and hemolytic components. The massive fish kills are possibly related to the hemolytic fraction (ILO 1984). A convenient nomenclature has been adopted (Poli et al, 1986), and uses the annotation PbTx-1-10 (which stands for *Ptychodiscus brevis* Toxin 1 through 10) to describe all of the known brevetoxins.

## 2. neural mechanism

Mice, birds and fish are all susceptible. The mouse LD50 is 0.20 mg/kg body weight (0.15-0.27) intraperitoneally. In human cases, the NSP concentration present in contaminated clams has been reported 30-18 MU/100g (ILO 1984).

The brevetoxins are lipid soluble polyethers with molecular weights around 900. They are eleven member heterocyclic oxygen-containing fused ring system culminating in an unsaturated lactone at one end and an unsaturated aldehyde at the other, differing by different substituents on carbon 41 (Viviani 1992).

The brevetoxins are depolarizing substances in the cholinergic systems which open membrane channels permeable to Na<sup>+</sup>, leading to Na<sup>+</sup> influx with profound effects on the Na channels. In some cases only the nerve is depolarized, and in others both the nerve and muscle (Baden 1983). This alters the membrane properties of excitable cell types in ways that enhance the inward flow of sodium ions. This current is blocked by external application of tetrodotoxin. In addition, neuromuscular blocks appear to due to the persistent sodium channel activation, although depletion of neurotransmitter from synapses may play a role. Brevetoxin A allosterically enhances sodium channel activation in the presence of toxin that binds to receptor site 2. (Gallagher 1980, Shinnick-Gallagher & Shinnick-Gallagher 1980, Baden 1983, Halstead 1988, Schantz & Johnson 1992, Viviani 1992).

Finally, it is believed that the respiratory problems associated with inhalation of these red tides are due to the opening of sodium channels which release acetylcholine and cause smooth tracheal muscle contraction, although only temporarily (Gallagher 1980, Baden 1983, Halstead 1988, Viviani 1992).

### 3. clinical presentation

The two forms which have been characterized in Florida are an acute gastroenteritis with neurologic symptoms following ingestion of shellfish, and a reversible upper respiratory syndrome following inhalation of aerosols of cells or toxins of *P. brevis* (Viviani 1992).

Ingestion of contaminated shellfish is followed in about 3 hours by paresthesias, hot/cold temperature reversal, nausea, diarrhea, vertigo, vomiting, mild diarrhea, pupil dilation, throat tightness or a choking sensation, and ataxia. There is no paralysis. The clinical course and the symptoms are relatively milder but similar to PSP or ciguatera. There is complete recovery within 2 days from ingestion. No human deaths have ever been reported with the Florida episodes, although the red tides are usually accompanied by extensive fish kills with significant commercial impact (Viviani 1992). Only one death from respiratory failure has ever been reported associated with a NSP like illness (Hemmert 1975, Morris et al, 1991, Viviani 1992).

The upper respiratory syndrome consists of conjunctival irritation, rhinorrhea, nonproductive cough and bronchoconstriction with the inhalation of the aerosol of cells or toxins of *G. breve*. It is rapidly reversible and may be unique to Florida (Baden 1983).

### 4. diagnosis

The diagnosis is based on the clinical scenario of persons becoming ill with gastrointestinal and neurologic symptoms

after eating shellfish. There is a mouse bioassay with crude toxic residue extracted with ethyl ether and a mosquito fish bioassay. In addition, recently HPLC methodology has been developed for the identification of the *P. Brevis* toxins (Baden 1983, ILO 1984, Halstead 1988).

#### 5. management and treatment

Gut decontamination and supportive care, particularly respiratory, are recommended for the treatment of NSP, as with PSP. Hemodialysis used in 2 cases did not provide a clear cut alteration in course (Brown & Shepherd 1992).

Use of particle filter masks or retreat to air conditioned environment will provide relief from the airborne irritation (Baden 1983).

As with many of the marine toxin induced diseases, the initial or index case(s) are often the tip of the iceberg. Therefore any cases of NSP should be reported to the appropriate public health authorities for follow up to ascertain other cases and to prevent further spread. And every effort should be made to obtain contaminated materials and their source (Morris et al, 1991).

The Florida Department of Natural Resources since the mid 1970s has run a control program with closure of shellfish beds commercially when *P. brevis* concentrations are greater than 5000 cells/liter until 2 weeks after with mouse bioassay testing. In addition, there is monitoring of red tides with their characteristic discoloration and massive fish kills, as well as reports of respiratory irritation (ILO 1984).

In the laboratory, *C. virginica* oysters accumulate *P. brevis* toxins in less than 4 hours in the presence of less than 5000 cells/ml of *P. brevis*, and the oysters then are able to "detoxify" 60% of this toxin in 36 hours when placed in *P. brevis* free sea water (Baden 1983). Canning does not decrease the NSP concentration in bivalves; commercial bivalves are usually safe to eat 1-2 months after the termination of a single bloom episode (Viviani 1992).

## D. Ciguatera Toxins

### 1. background & epidemiology

Ciguatera is a gastrointestinal illness with chronic neurologic manifestations reported worldwide (Bagnis et al 1979, Fernandez-Galiano 1982, Morris et al, 1982a, Morris et al, 1982b, Withers 1982, Baden 1983, Anderson et al, 1983, Bagnis et al, 1984a, ILO 1984, Ragelis 1984, Carmichael et al, 1986, Gillespie et al, 1986, Lewis 1986, Bagnis 1987, Lange 1987, Hokama 1988, Halstead 1988, Miller et al, 1991, Juranovic & Park 1991, Gollop & Pon 1992, Swift & Swift 1993, Bagnis 1993). It is caused by the consumption of contaminated fish and is not associated with red tides (Halstead 1988).

Ciguatera poisoning is the food borne illness most commonly caused by a marine toxin (Lange 1987, Miller et al, 1991). Several different toxins, singly and in combination, have been associated with Ciguatera poisoning, including ciguatoxin, maitotoxin, and scaritoxin. Ciguatoxin, is the toxin identified as that responsible for the majority of human illness known as Ciguatera Poisoning (ILO 1984, Carmichael et al, 1986, Sakamoto et al, 1987).

It has been reported since ancient times and it is found in tropical and subtropical areas around the world (from 35 degrees north to 35 degrees south latitude), with epicenters in the Caribbean and the Indo-Pacific islands (Lewis 1986, Lange 1987, Miller et al, 1991). The growth of an international fish market (especially exportation to industrialized countries), and massive tourism has resulted in Ciguatera Poisoning as a clinical entity being seen outside of the endemic tropical and subtropical areas (Lewis 1986, Anon 1986, Lange 1987, Morris et al 1990, Morris 1990, Lange et al, 1992). Unlike many of the other marine seafood-induced maladies already described, ciguatera often presents as large disease clusters, due to group consumption of a

single contaminated fish.

Ciguatera was mis-named for local Caribbean marine snail the *Livona pica* (L.) or 'cigua' (Steinfeld & Steinfeld 1974). Ciguatera was first reported in the West Indies by Peter Martyr de Anghera in 1511, in the Indian ocean (Mauritius) by Harmansen in 1601, in the Pacific ocean (New Hebrides) by De Quiros 1606 (Halstead 1967). In 1771 Portuguese biologist Parra in 1771 described "Siguatera" fish poisoning with symptoms of pallor, malaise, anorexia, arthralgias, diarrhea, vomiting, itching, dyspnea; there were few deaths and recovery in the majority of victims in 1 month. The surgeon, William Anderson, accompanying Captain James Cook in 1774 in the South Pacific reported a similar illness among the sailors after eating local fish (Poey 1866, Pearn 1989a). Captain Bligh, after the mutiny aboard the HMS Bounty on June 10, 1789 in an open boat described that he and his crew suffered a 'prodigious sickness' after eating fish (Steinfeld & Steinfeld 1974).

An estimated 10,000 to 50,000 people per year who live in or visit tropical and subtropical areas suffer from Ciguatera (Miller et al, 1991). Under-diagnosis and under-reporting (especially in endemic areas since people do not seek medical attention) make it difficult to know the true worldwide incidence of Ciguatera (Hanno 1981). CDC and others estimate that only 2-10% of cases are actually reported in the United States (Coleman 1990, Swift & Swift 1990, Bagnis 1993). It is the most common disease and nonbacterial food poisoning associated with consumption of fish in the US and territories (Ragelis 1984).

The reported incidence and prevalence of Ciguatera poisoning is variable throughout the world. In certain islands of the South Pacific, upto 43% of the population during one outbreak (Rodgers and Muench 1986) and in Puerto Rico, upto 7% of the residents has experienced at least one episode of Ciguatera in their lifetime (by telephone survey) (Holt 1984). In the United States, nearly half of the reported foodborne disease outbreaks of chemical origin are due to marine toxins, with ciguatoxin causing at least one third of these outbreaks; 90% of the reported cases of Ciguatera poisoning come from Florida and Hawaii (Hughes & Merson 1976, Lange 1987).

The social and economic impact in endemic regions is known for this marine toxin-induced illness (Bourdy et al, 1992,

Lewis 1986). In several endemic areas, the local fish are completely avoided as a food source. In Florida, the sale of barracuda (a major source of Ciguatera poisoning) is banned (Lawrence et al, 1980); in the Virgin Islands, many restaurants import fish due to the abundance of Ciguatera contamination (Payne & Payne 1977). Lewis (1986) in extensive anthropologic studies found that Ciguatera Poisoning in the South Pacific caused depression of both the local and exporting fishing industries and tourism, and had an indirect affect on human health due to avoidance of fresh fish consumption (despite its nutritional value). Many recent cases of Ciguatera reported in the medical literature have concerned imported cases in non-endemic areas, through tourism and fish importation (Johnson & Jong 1983, Saunders 1987, Sozzi et al, 1988, Hashmi et al, 1989, Schatz 1989, Sandor et al, 1990, Todd 1990, Biaggi 1991, Shelley 1991, Leung et al, 1992, Lange et al, 1992). In Canada, with an estimated 1000 cases per year due to tourism and food importation, the average medical cost was \$2470/case of ciguatera (Todd 1990).

Ciguatoxin, elaborated by the dinoflagellates of the genus *Gambierdiscus*, is a lipid soluble, heat stable and acid resistant neurotoxin (Scheuer et al, 1967, Asomata 1977, Carmichael et al. 1986, Sakamoto et al, 1987). The polyether structure has only recently been described (Yasumoto & Murata 1993). The toxin, causing no obvious adverse effects to the fish, can not be detected by smell or taste. Nor is it eliminated by cooking or other preparation procedures (Lewis 1986, Nellis & Bernard 1986). It reaches humans after being bioconcentrated in the food chain through the reef-feeding herbivores to the larger predacious fish which are eaten by humans. Over 400 reported species of fish have been found to carry ciguatera (Halstead 1988). Especially herbivore fish that feed on algae and coral reef detritus such as surgeon and parrot fish, and the large reef carnivores such as barracuda, moray eels, snappers, groupers, spanish mackerel have been reported. In general, larger size means more toxin, especially in the liver, intestines, ovaries and least of all, the muscle. The worst outbreaks reported have been with the consumption of moray eels (Halstead 1988, Halstead 1978, Shelley 1991).

Natural and human-made disturbances of the coral reef may lead to increases in ciguatera due to rapid recolonization by the dinoflagellate *G.toxicus* which appears to grow better after disturbance than under normal conditions (Anderson et al, 1983, Bagnis 1984a, Bagnis 1987, Viviani 1992). Increases have also been associated with military activities which disturb coral reef ecology such as nuclear test explosions and setting up of the infrastructure for these tests (Ruff 1989).

## 2. neural mechanism

As mentioned, Ciguatera has been reported after the consumption of fish contaminated by several different heat and acid stable polyether marine toxins, all elaborated by the dinoflagellate *G. toxicus*. The two most common toxins are Ciguatoxin and Maitotoxin, and they are some of the most lethal natural substances known. In mice, ciguatoxin is lethal at 0.45 ug/kg ip, and maitotoxin at a dose of 0.15 ug/kg ip. Oral intake of as little as 0.1 ug ciguatoxin can cause illness in the human adult (as an extrapolation from fish samples eaten) (Bagnis 1987, Brown & Shephard 1992).

Ciguatoxin, a lipid soluble substance, opens voltage dependant sodium channels in cell membranes which induces membrane depolarization. It causes prolonged symptoms indicate nerve blockage or damage requiring regeneration of nervous tissue Maitotoxin, water soluble, specifically increases the calcium ion influx through excitable membrane; this is not affected by tetrodotoxin or sodium. Usually Maitotoxin is less important since it is less present in fish. Scaritoxin is similar to Ciguatoxin. Okadaic Acid is a lipid soluble toxin with a LD50 210 ug/kg ip in mice; it is a sodium ionophore. Palytoxin is a water soluble polyether which causes severe tonic contraction of all muscle groups; it also strong skin irritant and potent tumor activation (Bagnis 1987, Schantz & Johnson 1992, Lange et al, 1992, Swift & Swift 1993).

The pharmacologic action of Ciguatoxin is due to its direct effects on excitable membranes. Its potent depolarizing action due to a selective increases in sodium permeability in the nerve cells and striated muscle can be counteracted by calcium ions and tetrodotoxin. The respiratory arrest induced by a lethal dose results mainly from depression of the central respiratory center. It causes biphasic cardiovascular response with hypotension and bradycardia (which can be antagonized with anticholinergics) followed by hypertension and tachycardia (which can be suppressed by adrenergic blockers). The response of smooth muscle to ciguatoxin is complex, depending upon the predominant autonomic innervation and postsynaptic receptor. It causes a potent release of endogenous norepinephrine from adrenergic nerve terminals and a potentiating effect on the post synaptic membrane (Ohizumi et al, 1981, Takahashi et al, 1982, Bagnis 1987, Hokama 1988).

Maitotoxin possesses a specific  $\text{Ca}^{2+}$  dependent action which causes a release of norepinephrine from rat pheochromocytoma cells. This action occurs in the absence of  $\text{Na}^{+}$  ions and in the presence of tetrodotoxin, precluding the participation of sodium channels; Maitotoxin appears to exert its effects on endogenous membrane calcium channels (Baden 1983).

Isolated rabbit ileal tissue was exposed to crude toxin; enterotoxic activity mediated by calcium was observed with no damage to intestinal mucosa. The authors conclude that the toxins involved in ciguatera fish poisoning directly stimulate intestinal fluid secretion without accompanying tissue damage and suggest that calcium acts as a "second messenger" mediating the process (Fasano et al, 1991).

Experiments by Cameron et al (1991a & 1991b) injecting toxic fish extract interperitoneally and studying nerve conduction in the ventral nerve in the tail of adult rats, showed significant slowing of mixed and motor nerve conduction velocities and F wave responses recorded. The motor and mixed nerve amplitudes were significantly reduced. Both absolute and supernormal periods were significantly prolonged with exaggeration of the supernormal response. The authors interpret these findings as suggesting that ciguatoxin acts on the mammalian nerve by prolonging sodium channel activation (Cameron et al, 1991a, Cameron et al, 1991b).

In human atrial trabeculae, Ciguatoxin-1 caused a large, sustained concentration dependent positive inotropy which was abolished by atenolol (beta 1 selective antagonist) and tetrodotoxin, and not eliminated by Mannitol (Lewis et al, 1992). In humans, orthostatic hypotension associated with Ciguatera fish poisoning was shown to be not due to volume depletion. Rather, it was due to low plasma catecholamine levels and marked pressor hypersensitivity to norepinephrine infusion. The hypotension and bradycardia reversed with atropine infusion and the heart rate after atropine plus propanol was normal. Therefore, the authors believe that orthostatic hypotension with Ciguatera poisoning is due to both parasympathetic excess and sympathetic failure (Geller & Benowitz 1992).

Pharmokinetic studies in a variety of animal species are on-going. These studies are attempting to establish dose

response, as well as metabolism and excretion of the toxin. Preliminary studies looking at both Ciguatoxin and its metabolites in the Toadfish animal model, have found very rapid metabolism of the toxin within a few minutes. The highest concentration of toxin metabolites was found in the Toadfish gallbladder, therefore it is important to consider feces as a major source of toxin excretion (P. Walsh, Rosenstiel School of Marine and Atmospheric Sciences (Miami), verbal communication). Further studies are in progress to examine the effect of Ciguatoxin on the inflammatory response and in antibody production.

### 3. clinical presentation

(Bagnis et al, 1979, Fernandez-Galiano et al, 1982, Bagnis 1984b, Anderson et al, 1983, Grant 1984, Lewis 1986, Bagnis 1987, Hokama 1988, Miller et al, 1991, Bagnis 1993).

The human disease entity of "Ciguatera Poisoning" is a direct result of the stimulation of the adrenergic and cholinergic nervous system due to the opening of the voltage-dependent sodium channels in the nerve cell membrane by the ciguatoxin (Gillespie et al, 1986, Lange 1987). The poisoning presents primarily as an acute neurologic disease manifested by a constellation of gastrointestinal (diarrhea, abdominal cramps and vomiting), neurologic (paresthesias, pain in the teeth, pain on urination, blurred vision, temperature reversal) and cardiovascular (arrhythmias, heart block) signs and symptoms within a few hours of contaminated fish ingestion. The pathneumonic symptom of Ciguatera intoxication is hot/cold temperature reversal although not all patients report this.

The attack rate has been reported to be 73%-100% with ingestion of contaminated fish, without any apparent age-related susceptibility (Lawrence et al, 1980, Engleberg et al, 1983, Czernichow et al, 1984). Acute fatality, usually due to respiratory failure, circulatory collapse or arrhythmias, ranges from 0.1% to 12% of reported cases; presently in the Pacific, the mortality is less than 1% (Bagnis et al, 1979, Craig 1980, Anderson et al, 1983, Withers 1982, Morris et al, 1982a, Morris et al, 1982b, Gillespie et al, 1986, Lange 1987, Kodama & Hokama 1989, Gollop & Pon 1992). Lethality is usually seen with ingestion of the most toxic parts of fish (ie. the liver, viscera, roe and other organs) (Hokama 1988).

The clinical picture may be variable among individuals, even with the same food source, different ethnic groups, and possibly with different types of fish and/or geographic location (Mills et al. 1988, Miller et al, 1991, Swift & Swift 1993). It appears that ciguatera from consumption of carnivore species may be more toxic than that from consumption of herbivores due to exposure to more than one toxin and/or transformation of the toxin(s) and/or an increased dose response (Bagnis 1968, Hokama & Miyahara 1986, Hokama 1988, Kodama & Hokama 1989, Miller et al, 1991). In Polynesia, Ciguatera is dominated and initiated by neurologic symptoms (90% of patients report paresthesias and dysesthesia), while reports from the Caribbean suggest that Ciguatera initially presents acutely as a gastroenteritis often with associated cardiovascular symptoms, with the gradual onset and dominance of neurologic symptoms over the first 24 hours. This may be due to different toxins mixtures elaborated by Caribbean and Polynesian *G. toxicus* (Bagnis 1968, Bagnis et al, 1979, Lawrence et al, 1980, Morris et al, 1982a, Morris et al, 1982b, Withers 1982, Holt 1984, Hokama & Miyahara 1986, Hokama 1988, Miller et al, 1991, Blythe et al, 1992, Bagnis 1993). Regardless, it is the neurologic symptoms which persist often weeks to months after the initial illness.

The symptoms of Ciguatera poisoning, especially the paresthesias and weakness, can persist in varying severity for weeks to months after the acute illness. For example, in a recent epidemic in Iles Saintemedian, the mean duration of symptoms reported was 3 weeks and 30% of the victims had longterm sequelae (Czernichow et al. 1984). In the Pacific, chronic neurologic symptoms have been reported even upto 25 years (Halstead 1988). Prolonged itching due to chronic Ciguatera can present as a dermatologic disease when it is really due to ciguatera paresthesias (Schiazza et al. 1987). Chronic ciguatera can also present as a psychiatric disorder of general malaise, depression, headaches, muscular aches, and peculiar feelings in extremities for several weeks (Lipki 1989). It is reported that those with chronic symptoms seem to have recurrences of their symptoms with the ingestion of fish (regardless of type), ethanol, caffeine, and nuts 3 to 6 months from initial ingestion (Lawrence et al, 1980, Anderson et al, 1983, Bagnis 1987, Hokama 1988, Ruff 1989, Lange et al, 1992, Germain & Paul 1981, Brown 1992).

Ciguatera can be sexually transmitted as was evidenced in the report of painful ejaculation of the affected male followed by dyspareunia in previously unexposed and unaffected female partner (Lange et al, 1989, Lange et al, 1992). Its toxins can apparently also cross the placental barrier. With exposure of the mother, premature labor and spontaneous abortion have been

reported. There have been reports of increased fetal movement followed by decreased fetal activity, although ultimately resulting in normal child delivery, with exposure in the second trimester (Senecal & Osterhol 1991, Rivera-Alsina et al, 1991). Pearn et al (1982) reported that exposure near term resulted in the birth of a child with left sided facial palsy and possible myotonia of both hands; this completely resolved within 10 months. Finally, ciguatera has been reported to be transmitted by breastmilk (Thomas 1989, Blythe & de Sylva 1990).

In addition to the nerve conduction results described above in cases of acute and chronic Ciguatera, EMGs can be normal even with on-going symptoms, as well as lumbar puncture and EEGs (Chretien et al, 1981, Casanova et al, 1982). Biopsy proven polymyositis subsequently developed in 2 patients severely poisoned by ciguatera (Stommel et al, 1991). Atrophy can accompany the neuropathy of chronic Ciguatera especially in the leg muscles. Finally, there are reports of inverted t waves on EKG taken acutely which reverted to normal with clinical improvement (Hanno 1981).

In humans, the neurologic effects associated with Ciguatera are due to effects on both the central and peripheral nervous systems. The fact that Ciguatera does have probable central nervous effects is evidenced by reports of headache, hallucinations, memory disturbances, and in severe cases convulsions and coma (Bagnis et al 1977). The peripheral nerve effects have been better studied although with variable results. Electrophysiologic studies in 15 cases of acute ciguatera in Australia on the sural and common peroneal nerve showed significant slowing of sensory conduction velocity and prolongation of the absolute refractory, relative refractory and supernormal periods, which suggest that ciguatera causes an abnormally prolonged sodium channel opening in nerve membranes (Cameron et al, 1991b). In 9 cases of ciguatera from barracuda, Ayyar et al (1977) found distal slowing in the median, ulnar and sural nerves without significant decrease in nerve amplitudes, and concluded that the site of action was the myelin sheath rather than the axon. Jones et al (1980) found no significant disturbance in nerve conduction in one case 7 days after onset of symptoms. Allsop et al (1986) described 3 severe cases with slowing of motor conduction velocity in all 3. One case had significant slowing of motor and sensory velocities and distal latencies, as well as sensory amplitudes and F wave latencies. Biopsy showed edema in the adaxonal layer of the Schwann cell cytoplasm, axonal compression and vesicular degeneration of the myelin, but the authors postulated that scaritoxin rather than ciguatoxin was involved since there was

reported cerebellar involvement. Sozzi et al (1988) found polyneuropathy secondary to marine fish ingestion in Thailand in a person with a prior history of Multiple Sclerosis, sparing tendon reflexes. They report a demyelinating polyneuropathy with intramyelinic edema or edema between myelin and axon with compression of the axon.

In the differential diagnosis of Ciguatera, poisoning with the other marine toxins, especially NSP and PSP should be considered since dysesthesias with nausea, vomiting and diarrhea are the presenting symptoms. Obviously the history of fish versus shellfish consumption should help to differentiate. Type E botulism with ingestion of smoked fish, Scombrotoxicosis and even Eosinophilic meningitis from helminthic infection of *Angiostrongylus cantonensis* from ingestion of raw mollusks, crabs and certain fish should be considered. Finally, poisoning with organophosphates pesticides can present initially with a similar clinical picture except for the exposure history (Johnson & Jong 1983, Miller et al, 1991).

#### 4. diagnosis

Using a household pet or even elderly relative as a simple bioassay was and may still be practiced in many island communities (Lewis 1986, Cooper 1964). Otherwise, only expensive ponderous bioassays in such animals as the mongoose, rat and cats were available for screening Ciguatera-contaminated fish until ten years ago (Lange 1987, Miller et al, 1991). The mouse bioassay, while it remains the standard diagnostic tool, does not distinguish between ciguatera and scaritoxin. Then the Hokama enzyme immunoassay stick test was introduced, predominantly in Hawaii (Hokama 1985, Hokama 1988). Appropriately, this test has a high sensitivity and low specificity; this has resulted in the rejection of as much as 75% of the fish catch and a resultant marked decrease in reported cases of Ciguatera Poisoning in Hawaii. Since (at least in the United States) the effects of contaminated fish on the marketplace are more harmful from a cost-benefit point of view than the loss of false positive contaminated fish, this is considered a successful program.

Over the past few years, radioimmune (RIA) or enzyme linked immunosorbent (ELISA) assays have been developed to investigate Ciguatera (Hokama et al, 1977). Emerson et al (1983) using counter-immunoelectrophoresis disclosed precipitin lines

with toxic fish extracts and effectively discriminated between samples compared with human and mouse bioassay. However putative immune and nonimmune serum gave equally clear precipitin reactions with toxic extracts therefore the authors could not conclude that they had located a specific antibody. Then Trainer et al (1990, 1991) developed an assay which can measure Ciguatoxin qualitatively and potentially quantitatively in fish and possibly human fluids. These assays use counts or color changes to provide a quantitative analysis of toxin burden. They consist of antibodies which are epitope specific to the toxin and/or its metabolites. Baseline sensitivity and specificity information is being gathered for these assays comparing results in contaminated fish to the traditional assays applied to same fish. Further work involves the application of these assays to human fluids from persons who have eaten assay-positive fish.

#### 5. management and treatment

Medical treatment has been to a large extent symptomatic; a variety of agents, including vitamins, antihistamines, anticholinesterases, steroids and tricyclic antidepressants, have been tried with limited results (Germain & Paul 1981, Gillespie et al, 1986, Lange 1987, Miller et al, 1991). Gut emptying and decontamination with charcoal is recommended acutely although often the severe ongoing vomiting and diarrhea prevents this. Atropine is indicated for bradycardia, and dopamine or calcium gluconate for shock. It is recommended that opiates and barbiturates be avoided since they may cause hypotension, and opiates may interact with maitotoxin (Brown 1992).

With apparent considerable success, at least acutely, mannitol infusions have been used (Palafox et al, 1988, Pearn et al, 1989b). Palafox et al (1988) administered 1 gm/kg of 20% mannitol at a rate of 500 mL/h "piggybacked" to an iv infusion of 5% dextrose in Ringers lactate or saline solution at 30 mL/h or more depending on fluid requirements with complete reversal of symptoms in the majority of patients tested. Subsequent reports have affirmed his success although mannitol appears to be most effective in completely relieving symptoms when given within the first 48 hours from ingestion (Williamson 1980, Frenette et al, 1988, Palafox et al, 1988, Pearn et al, 1989b, Stewart 1991, Ayyar et al, 1991, Bagnis 1992, Blythe et al, 1992, Blythe et al, 1993, Swift & Swift 1993). It is hypothesized that mannitol may act as a scavenger molecule for hydroxyl radicals since ciguatoxin possesses

hydroxyl groups, and/or as an osmotic agent by drawing fluid from tissues, especially to decrease the accumulation of excess fluid within Schwann cells and axonal cytoplasm which are some of the effects of ciguatera (Gillespie et al, 1986, Pearn et al, 1989b, Swift & Swift 1993). It is possible that mannitol dissociates the ciguatoxin molecule from its binding site due to its osmotic properties since experiments with guinea pig atria had permanent reversal of ciguatoxin effects with the addition of mannitol, while tetrodotoxin and lidocaine were only temporary (Swift & Swift 1993).

Amitriptyline (25 to 75 mg bid) does seem to have some success in relieving the symptoms of chronic Ciguatera, such as fatigue and paresthesias (Germain & Paul 1981, Bowman 1984, Davis & Villar 1986, Calvert et al, 1987, Lange et al, 1988, Lipkin 1989, Lange et al, 1992, Berlin et al, 1992). It is possible that nifedipine may be appropriate as a calcium channel blocker to counteract the effects of maitotoxin (Calvert et al, 1987, Miller et al, 1991, Lange et al, 1992). Although others report no benefit, Donnet et al (1987) reported that anticholinesterases may work for myasthenic chronic symptoms in a case from Antilles, both subjectively and by improvement in nerve conduction studies (Donnet et al, 1987). Finally, there are over 64 different local remedies including medicinal teas used in both the Indo-Pacific and West Indies regions; many of these remedies are symptomatic for gastroenteritis, some are emetics, some are "antidotes" and some are analgesics (Dufva et al, 1976, Lewis 1986, Swift & Swift 1990, Bourdy et al, 1992).

None of these treatments have been evaluated in a controlled clinical trial with the exception of a controlled trial of Mannitol versus glucose plus vitamin B6 and calcium gluconate for treatment of acute Ciguatera (Bagnis 1992), so that their true efficacy is impossible to determine. The inaccuracy of the diagnosis of Ciguatera poisoning, which has relied heavily on the clinical history, has lead to confusion with a number of other clinical entities including other marine toxin diseases and organophosphate poisoning.

As mentioned above, there appears to be a sensitivity to certain foods after ciguatera poisoning and these should be avoided for 3 to 6 months after the illnesses. In addition, there is no immunity to this illnesses and recurrences of actual ciguatera in the same individual appear to be worse than the initial illness (Brown 1992).

As with many of the marine toxin induced diseases, the initial or index case(s) are often the tip of the iceberg. Therefore any suspected cases of Ciguatera should be reported to the appropriate public health authorities for follow up to ascertain other cases and to prevent further spread. And every effort should be made to obtain contaminated materials and their source (Bagnis 1978, Lawrence et al, 1980, Anderson et al, 1983, Lewis 1986, Maharaj 1986, Kodama & Hokama 1989, Coleman 1990, Todd 1990). The Pan American Health Organization has recommended plan to decrease the occurrence of Ciguatera in tourists and imported cases out of endemic areas. This plan includes education of persons living in and traveling to endemic areas, as well as surveillance of the fish and of the epidemiology, with international cooperation (Todd 1990).

Obviously persons who live in or travel to endemic areas should never eat barracuda or morey eel, and should be cautious with grouper and red snapper (Lange et al, 1992). Since there is no reliable way to "decontaminate" or even to distinguish contaminated fish by smell or appearance, at a minimum, people should be advised to avoid the viscera of any reef fish as well as avoiding consuming unusually large predacious reef fish especially during the reproductive season (Lewis 1986, Halstead 1988). It is hoped that new diagnostic methods both in fish and in humans will lead to greater prevention and understanding of Ciguatera in the future.

E. Diarrheic Shellfish Poison (DSP)

1. background & epidemiology

DSP is a gastrointestinal illness without neurologic manifestations reported worldwide (ILO 1984, Halstead 1988, Aune & Yndstad 1993). It is caused by the consumption of contaminated shellfish (Halstead 1988).

The first reported cases of DSP were in the Netherlands in the 1960s, followed by similar reports in the late 1970s from Japan (Aune & Yndstad 1993). Since then, more than 1300 cases have been reported from Japan, with the peak season from April

to September. Other outbreaks have been reported in Europe and South America as well as the Far East. In Spain, over 5000 cases were reported in 1981; In France in 1984 and 1986, over 2000 cases were reported each year and over 300 cases were reported in Scandinavia in 1984 (Asomata et al, 1978, Yasumoto et al, 1980, Viviani 1992, Aune & Yndstad 1993). Mussels exported from Denmark to France caused DSP poisoning in over 400 people in 1990 (Hald et al, 1991). Finally in 1991 DSP was reported in over 100 people in Chile; in 1992, DSP was detected in toxic concentrations in shellfish beds in Uruguay (Lembeye et al, 1993, Mendez 1992, Aune & Yndstad 1993). Although DSP is reported worldwide, the most highly affected areas appear to be Europe and Japan (Aune & Yndstad 1993).

The causative organisms are the marine dinoflagellates *Dinophysis* (especially *D. fortii* and *D. acuminata*) and *Prorocentrum*, although there is an uneven distribution among species and location of toxin production. These dinoflagellates are widely distributed, but do not always form red tides. The associated toxins produced by the *Dinophysis* dinoflagellates are okadaic acid and its derivatives (dinophysiotoxins), the polyether lactones (pectenotoxins), and recently, yessotoxin; there are at least 9 total toxins produced by these dinoflagellates. This group of toxins was discovered in the late 1970s (Asomata et al, 1978, Yasumoto et al, 1980, ILO 1984, Lee 1989). Neither the pectenotoxins nor yessotoxin cause diarrhea, and therefore their potential health threat to human consumers needs to be clarified (Aune & Yndstad 1993).

## 2. neural mechanism

*D. fortii* at levels of 200 cell/litre in mussels and scallops becomes toxic for humans; the minimal amount of DSP toxins required to induce disease in humans was 12 MU (Asomata et al, 1978, Aune & Yndstad 1993).

Okadaic acid, dinophysiotoxin 1 and 3 are acidic, while there is another neutral group of toxins which are polyether lactones named pectenotoxins and yessotoxin. Diarrhea is caused in mice when the acidic component of okadaic acid is injected interperitoneally. Pectenotoxin 1 causes liver damage in mice under similar circumstances. Both the pectenotoxins and yessotoxin are lethal in mice with ip injection (Viviani 1992, Aune & Yndstad 1993).

Okadaic acid is lipophilic. It is a potent inhibitor of protein phosphorylase phosphatase 1 and 2A in the cytosol of the mammalian cells that dephosphorylate serine and threonine. It probably causes diarrhea by stimulating the phosphorylation that controls sodium secretion by intestinal cells similar to *Vibrio cholerae*, although by a different mechanism. Okadaic Acid also acts through the variations of cellular concentration of the Ca<sup>2+</sup> second messenger. It strongly increases the Ltype inward Ca<sup>2+</sup> current in isolated guinea pig cardiac myocytes. Finally, okadaic acid functions not only as a tumor promotor (promoter of skin tumor in the mouse using DMBA as the initiator), but it is also capable of reversing cell transformation in some oncogenes (Yasumoto et al, 1989, Viviani 1992, Aune & Yndstad 1993).

### 3. clinical presentation

This is a self-limited diarrheal disease without known chronic sequelae. There is no evidence of neurotoxicity and no fatal cases have ever been reported (Halstead 1988, Viviani 1992).

Diarrhea was the most commonly reported symptom (92%), closely followed by nausea (80%) and vomiting (79%), with onset 30 minutes to 12 hours from ingestion. Complete clinical recovery is seen even in severe cases within 3 days (Asomata et al, 1978, Viviani 1992, Aune & Yndstad 1993).

### 4. Diagnosis

A mouse bioassay using an intraperitoneal injection of toxin extracts with a 24 hour waiting period is used in Japan and shellfish with DSP toxin levels greater than 50 MU/kg are banned; similar surveillance systems have been established in the European countries (ILO 1984, Viviani 1992, Aune & Yndstad 1993). An HPLC method for detection of DSP toxins is available and used in Sweden for monitoring purposes (Lee et al, 1987).

### 5. management and treatment:

Treatment is symptomatic and supportive with regards to short-term diarrhea and accompanying fluid and electrolyte losses. In general, hospitalization is not necessary; fluid and electrolytes can usually be replaced orally. Other diarrhetic illnesses associated with shellfish consumption, such as bacterial or viral contamination should be ruled out (Aune & Yndstad 1993).

As with many of the marine toxin induced diseases, the initial or index case(s) are often the tip of the iceberg. Therefore any suspected cases of DSP should be reported to the appropriate public health authorities for follow up to ascertain other cases and to prevent further spread. And every effort should be made to obtain contaminated materials and their source.

#### F. Amnesic Shellfish Poisons (ASP)

##### 1. background & epidemiology

ASP is a newly identified marine toxin disease, first reported from Canada and later identified as a continuing problem in Washington State and Oregon (Hungerford 1989, personal communication J. Wekell, NMFS Seattle). After an initial gastroenteritis with neurologic symptoms, some persons with ASP develop apparently permanent neurologic deficits, especially dementia. It is caused by the consumption of contaminated shellfish (Perl et al, 1990, Teitelbaum et al, 1990, Viviani 1992).

At the end of November 1987, 153 cases of acute intoxication after ingestion of toxic mussels were reported in Canada associated with the marine organism diatoms (Wright et al, 1989); in late 1991, domoic acid was found in anchovies, bivalves and crustaceans in California and Washington State (Hungerford 1993). Domoic acid, a relatively rare neurotoxic amino acid, was found in the marine pennate diatom, *Nitzschia pungens* Grunow forma multiseries Hasle. Samples in the Cardigan river estuary were found to be associated with domoic acid levels as high as 1% dry weight following the outbreak. Contaminated mussels had digestive glands engorged with *N. pungens* (Subba Rao et al, 1988, Perl et al, 1990).

It has been suggested that this bloom of the pennate diatom, *Nitzschia pungens*, may have been related to fertilizer run-off from extensive tobacco farming in the area (Viviani 1992).

## 2. neural mechanism

Domoic acid, and its co-existing natural chemical analogs act as a potent excitatory neurotransmitter. It is heat-stable and similar to its biochemical analogues, kainic acid and glutamic acid and binds at the same receptor site in CNS. Lesions in human brain, especially in the hippocampus, have been reported in the ASP cases which are similar to those seen in rats after kainic acid iv administration. When rats are exposed experimentally to domoic acid and its analogues, they get limbic seizures, memory and gait abnormalities, and degeneration of the hippocampus. In animals, domoic acid is three times more potent than kainic acid and 30-100 more potent than glutamic acid (Perl et al, 1990).

An estimated concentration of 200 ug/g wet weight domoic acid appeared to affect some human consumers (Viviani 1992).

Recent work by Novelli et al (1992) demonstrated that domoic acid from mussels is more neurotoxic for cultured human neurons than purified domoic acid. This increase is believed to be due to domoic acid potentiation, even in subtoxic amounts, of the excitotoxic effect of glutamic acid and aspartic acid. Glutamic and aspartic acids are present in high concentrations in mussel tissue. This neurotoxic synergism may occur through a reduction in the voltage dependent Mg<sup>2+</sup> block at the NMDA receptor associated channel, following activation of non-NMDA receptors by domoic acid (Novelli et al, 1992).

In humans, domoic acid appears to cause a non-progressive acute neuropathy involving anterior horn cells or a diffuse axonopathy predominantly affecting motor axons. The acute neuronal hyperexcitation syndrome presumably results from the stimulus of central and possibly peripheral neurons, followed by chronic loss of function in neural systems susceptible to

excitotoxic degeneration (ie. hippocampus and anterior horn cells of spinal cord) (Teitlebaum et al, 1990).

### 3. clinical presentation

Acute symptoms included vomiting, diarrhea, and in some cases, there followed confusion, loss of memory, disorientation and even coma. The acute symptoms were mild compared with PSP (Wright et al, 1989). Permanent neurologic sequelae, especially cognitive dysfunction, were most likely in persons who developed neurologic illness with 48 hours, males, in older patients (> 60 yrs), and in younger persons with pre-existing illnesses such as diabetes, chronic renal disease and hypertension with a history of transient ischemic attacks. Three elderly patients died (Wright et al, 1989, Perl et al, 1990).

Perl et al (1990) received 250 reports with 107 patients fitting the case definition. In the 99 patients who participated, the acute symptom frequencies were the following: vomiting (76%), abdominal cramps (50%), diarrhea (4%), severe headache (43%), and loss of short-term memory (25%). Acutely, the patients had headache, hyporeflexia, hemiparesis, ophthalmoplegia and abnormalities of arousal ranging from agitation to coma; in addition; seizures and myoclonus were observed acutely, especially around the face. Nineteen people required hospitalization with 12 admitted to intensive care units due to seizures, coma, profuse respiratory secretions or unstable blood pressure. Male sex and increasing age were associated independently with the risk of hospitalization and subsequent memory loss. Three patients died directly and one died indirectly from the intoxication, with neuronal necrosis or cell loss and astrocytosis especially in the hippocampus and amygdaloid nucleus noted on autopsy.

No domoic acid was identified in the blood serum or cerebral spinal fluid of 17 patients over 2 days from ingestion and of 2 controls. Short term elevations of serum creatine and BUN were reported (Perl et al, 1990, Teitlebaum et al, 1990).

Teitlebaum et al (1990) studied 14 persons with severe neurologic disease using a standardized battery of neuropsychologic testing, MRI, CT, EEG, Electromyography, and PET. In addition, neuropathology evaluation was obtained of 4 persons who died. In neuropsychological testing performed several months after the acute episode, 12/14 persons had severe

antegrade memory deficits with relative preservation of other cognitive functions. 11/14 persons had clinical and electromyographic evidence of pure motor or sensory motor neuronopathy or axonopathy. The PET results in 4/14 persons showed decreased glucose metabolism in the medial temporal lobes. The neuropathology for the 4 fatal cases, revealed neuronal necrosis and loss, predominantly in the hippocampus and amygdala.

All 14 with severe neurologic disease reported confusion and disorientation within 1.5 to 48 hours after consumption. The maximal neurologic deficits were seen 4 hours post ingestion of least affected and 72 hours in those most affected, with maximal improvement 24 hours to 12 weeks post ingestion. Acute coma was associated with the slowest recovery. Seizures ceased by 4 months but were frequent upto 8 weeks (Teitlebaum et al, 1990).

Relative preservation of intellect and higher cortical function appears to distinguish this disease from Alzheimer's Disease, and the absence of confabulation with well preserved frontal lobe function is not typical of Korsakoff's syndrome (Teitlebaum et al, 1990).

#### 4. diagnosis

The mouse assay used for ASP testing is the same as for PSP. The relative potency of ASP toxins appear to be less than PSP. In addition, involuntary scratching of shoulders with hind legs by the mice was noted and is not typical of PSP (Perl et al, 1990). HPLC analysis can quantify domoic acid from contaminated shellfish in ASP episodes (Lawrence et al, 1989).

#### 5. management & treatment

At this point, the treatment of ASP is symptomatic and supportive. Teitelbaum et al (1990) noted that the seizures respond to iv diazepam and phenobarbital. Three patients were resistant to dilantin for seizure control (Teitelbaum et al, 1990).

As with many of the marine toxin induced diseases, the initial or index case(s) are often the tip of the iceberg. Therefore

any suspected cases of ASP should be reported to the appropriate public health authorities for follow up to ascertain other cases and to prevent further spread. And every effort should be made to obtain contaminated materials and their source.

Since an estimated concentration of 200 ug/g wet weight domoic acid appeared to affect some consumers, with a safety factor of 0.1 applied, Canada has set a concentration of domoic acid of 20 ug/g wet weight above which shellfish commercial operations should be closed (Viviani 1992). Finally, this epidemic has lead to new attention to the diatoms, especially the appearance of the mucilage from diatoms with species of *Nitzschia* (Wright et al, 1989).

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