SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM

Papeete, French Polynesia, 16th - 22nd August, 1968

R E P O R T

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INTRODUCTION

This Seminar on Ichthyosarcotoxism was the first international meeting on the subject to be held in the South Pacific area. It was convened as the result of recommendations from the Seminar on Health Problems of Coral Atoll Populations (Tarawa, May 1967), and a request from French Polynesia.

As ichthyosarcotoxism is widespread and important throughout the area it serves, the South Pacific Commission has aided research and collected material for a period of eighteen years. Recent progress in research into the problem, with all its ramifications, indicated that a conference of specialists involved was very desirable so that each could exchange information and present this in collated form for the benefit of territories involved and for those active in research.

The Seminar was held at the "Ecole Normale de Tipaerui", Papeete, Tahiti, from the 16th to the 22nd of August 1968. After a formal opening by the Secretary-General of French Polynesia at a ceremony in the Council of Government Meeting Hall, Dr E. Poyet, Director of Health of French Polynesia, was elected as Chairman, with Professor A.H. Banner of the University of Hawaii, and Professor Y. Hashimoto of the University of Tokyo as Vice Chairmen; Mr M. Legand and Dr P. Helfrich were elected as French and English rapporteurs.

The purpose of the Seminar was the exchange of current information on all aspects of ichthyosarcotoxism, its clinical manifestations, its epidemiology, the nature, origin and pharmacological actions of toxins involved, its medical treatment and its economic and nutritional implications. In addition, the Seminar aimed at providing a wider understanding of the subject and at providing a stimulus for further research.

Participants represented all the major specialities involved and attended from Australia, Cook Islands, France, French Polynesia, Hawaii, Japan, New Caledonia, United Kingdom, and United States of America. Formal discussions were supplemented by visits to medical and fisheries research centres in Tahiti and Rangiroa as well as visits to reef areas where toxic species exist in Tahiti and Moorea.

Free discussion and the co-operation of participants resulted in a successful and stimulating Seminar which emphasised that, while some progress has been made, there still remains a great deal of research to be undertaken.

PROCEEDINGS

1. Definition

Basically the term ichthyosarcotoxism means fish flesh poisoning. It encompasses many differing varieties of intoxication which have been delineated either on the basis of the clinical picture produced or of the species of causative fish. Many species, many toxins are involved and precise definition or categorisation is not possible in the present state of knowledge. However, there appears to be broad differentiation between characteristic illnesses which appear following ingestion of diverse species of toxic fish and illnesses which occur following ingestion of particular species. In both categories, any portion of the fish may give rise to intoxication and this intoxication is not the result of accidental contamination by bacterial food pathogens. Fishes involved are those which inhabit tropical reef waters.

1.1 Ichthyosarcotoxism as the result of ingestion of diverse species of toxic fish

1.1.1 Ciguatera. Ciguatera is a clinical syndrome characterised by disturbances, in varying proportions, of the neurologic, cardiovascular, gastrointestinal and skeleto-muscular systems. Considerable variation in the syndrome exists but that most constant and characteristic is disturbance of the neurologic system manifested by dysthenia on contact with cold objects, myalgia and pronounced muscle weakness.

Most tropical fish poisoning falls into this category.

1.1.2 Hallucinatory fish poisoning. Hallucinatory fish poisoning is a clinical syndrome characterised by psychologic disturbances of hallucination and depression. Gastrointestinal disturbance may occur.

1.2 Ichthyosarcotoxism found in taxonomic groups

1.2.1 Elasmobranch poisoning. From ingestion of sharks, skates and rays.

1.2.2 Clupeoid poisoning. From ingestion of sardines and sardine-like fishes.

1.2.3 Scombroid poisoning. From ingestion of tuna and mackerel-like fishes.

1.2.4 Tetraodon poisoning. From ingestion of puffers and puffer-like fishes.

As ciguatera is the most commonly occurring form of fish poisoning in tropical areas, discussion will deal with this subject unless otherwise stated.

2. Importance

While, for most territories, no accurate figures are available on the social and economic impact of ichthyosarcotoxism, a recent survey in Tahiti provides an indication of its extent. One criterion of the impact of ciguatera is an assessment of the loss of working days by an individual.

It was established that the period during which the person affected was unable to work normally varied from two to seven days, and that, in extreme cases, this might extend to four weeks. The latter duration applied to serious cases generally treated at home with traditional medication. The period of absence from work was dependent on the severity of the illness which in turn varied according to the species involved.

A total of 6,580 working days of inactivity resulted from ichthyosarcotoxism in the year under study. Unfortunately it was not possible to distinguish between wage-earners and others but the latter were definitely more numerous in distant rural areas. It may be assumed with good reason, however, that the rural population has suffered greater damage, even though it is composed mainly of individuals who are not wage-earners, since any work stoppage always represents a total loss to them.

The repercussions in the economic field mainly concern the fishing industry. The supply of fish, whether or not it be provided through sales centres, normally covers the needs of the population. When the toxicity of fish increases there is a risk that this balance established between supply and demand will be destroyed, either through the progressive extension of prohibition measures which already affect certain reef species, or through an attitude of excessive mistrust adopted by the consumers themselves towards perfectly edible fish.

3. Evolution of fish poisoning

Fluctuations in the geographical distribution and intensity of ciguatera fish poisoning have been experienced in several areas of the Pacific. An examination of the available information suggests a sequence of events that typify an outbreak or marked increase in cases of ciguatera in a given area. The phenomena triggering such outbreaks are largely unknown; however, there is a hypothesis which suggests mechanisms whereby the sequence of events in fluctuations in incidence of ciguatera might be explained, i.e. that the toxin was produced by reef organisms, probably algae or organisms living on algae, which undergo unusually rapid growth under certain conditions. These toxins contained or produced by reef organisms, consumed by herbivorous fishes, are thus introduced into the food chains of numerous other fishes. It is suggested that at least one of the prerequisite conditions affecting algae could be the exposure of new surfaces on the submarine substrata as the result of blasting, shipwreck or natural disturbance.

According to data on fluctuations of ciguatera available from islands and archipelagoes of the Indo-Pacific, there appears to be three categories:

I - pattern of intoxications observed in areas with no previous history of ciguatera;

II - pattern of intoxications in areas with a continuous history of ciguatera at a low level, with periodic marked increases in ciguatera;

III - pattern of intoxications at a sustained low level with well-defined geographical limits and species involved.

Ciguatera is known to have occurred in the 16th century in the Indian Ocean and New Caledonia; more recent reports attest to its presence in the South-Central and North-Western Pacific and evidence exists that there has been a gradual spread in a North-Easterly direction. In 1939, it spread to the Line Islands, and in 1957 the first case was reported from the ingestion of reef fish in Hawaii.

4. Geographical distribution and epidemiological factors in ciguatera

Ciguatoxic fishes are widely distributed among oceanic islands in the tropical Indo-Pacific and in the Caribbean. They generally occur only around islands within 30° of the Equator and rarely along the coasts of continents with the exception of Australia. The large islands of the Philippines and Indonesia appear to be free of ciguateric fishes.

The species involved in ciguatera fish poisoning include a variety of reef fishes normally utilized as food. The most common are the acanthurids (surgeon fishes), the lutjanids (snappers), serranids (groupers), carangids (jacks, pompanos), scarids (parrot fish), and sphyraenids (barracuda). Ciguatera rarely occurs in tutas or other pelagic species.

Ciguatera may be prevalent in fishes from certain islands or certain parts of islands; the geographic range may expand and contract; the number of toxic species may fluctuate and the toxicity of species may vary in intensity but knowledge of the dynamics of these changes is little understood as it is based upon incomplete data from scattered reports. Some evidence exists that cyclical increases in ciguatera occur with a peak of outbreaks and subsequent decline to a low level for a prolonged period. During the periods of increase, more species of fish become toxic, and the level of toxicity of individual fish increases. In the decline, compulsive carnivorous fish show persistence of toxicity for a longer time than other species. This persistence is aggravated by fishing practices in some areas where gut, liver and waste are dumped at the sea where the fishes have been caught, thus producing cyclical transmission of toxins through scavenger fish.

The incidence of ciguatera is most marked in populations dependent on fish protein, thus tending to affect lower socio-economic groups. However, no stratum of society is immune and, where toxic fish species exist, the entire population is considered to be at risk.

5. Clinical picture

Ciguatera presents a polymorphous clinical picture with a few characteristic features which enable ready diagnosis to be made.

The full clinical picture is one of toxic polyneuritis in association, to a greater or lesser extent, with three other types of disturbance: gastrointestinal, cardiovascular and algic. General symptoms of malaise, asthenia and chills frequently appear and there have been reports of oliguria and haematuria. Asthenia may be severe enough to make walking difficult and may persist for a long period. Laboratory investigations are unproductive.

The most complete form with its various syndrome - neurologic digestive, cardiovascular and algic with repercussions on the general condition - is adopted as typical for purposes of description.

5.1 Incubation

The incubation period varies with the individual, the species of fish and the quantity of toxic flesh ingested, but it is generally short, of about two to twenty hours duration. It may be as short as thirty minutes in severe cases.

5.2 Onset

The first symptom to appear is paresthesia with prickling about the lips, tongue and nose, together with a tingling sensation in the extremities of the limbs. At the same time, a state of malaise accompanied by facial congestion and cold perspiration appears. Nausea, heralding digestive upset, may occur concomitantly.

This initial period lasts approximately two hours.

5.3 Established case

5.3.1 Neurological system

Paresthesia affecting mainly sensitivity to cold objects, with painful tingling of the extremities on contact with cold.

Superficial hyperesthesia with sensations of burning or electric discharge.

Mydriasis.

More rarely, paresis localised in the lower limbs with reduced knee and ankle reflexes.

Persistent pruritus. This may be severe.

5.3.2 Gastrointestinal system

Symptoms appear early. Nausea is rapidly followed by slight vomiting, then repeated attacks of abundant, watery diarrhoea accompanied by abdominal cramp and colic.

These symptoms generally subside within twenty-four hours, leaving the patient asthenic and dehydrated.

5.3.3 Cardiovascular system

Slow pulse rate; between 35 and 50 beats per minute, often irregular.

Muffled heart-beats.

Reduced blood pressure.

Electrocardiography shows disturbances in the rhythm of regular sinus bradycardial type.

5.3.4. Skeleto-muscular system

Arthralgia localised mainly in the large joints: knee, ankle, shoulder, elbow.

Myalgia affecting, in particular, thigh and leg muscles.

Individual cases exhibit differing proportions of each component of the clinical picture and it is only in those most severely affected that all components appear.

5.4 Progress and Prognosis

The condition is rarely fatal but deaths have been reported. Generally, the cardiovascular symptoms subside within a few days but paresthesia, asthenia and pruritus may persist much longer with some residual incapacity over a period of some months.

6. The toxin, its isolation, identity and properties

6.1 Evidence that a multiplicity of toxins are involved in ichthyosarcotoxism has been increasingly accumulated and at least seven different toxins have been demonstrated in toxic fishes.

A fat-soluble toxin, probably identical with ciguatoxin, has been found in the muscle of *Lutjanus bohar* and *L. monostigma*. This toxin may be a principal one in ciguatera.

A water-soluble toxin causing transient vomiting in cats has been found and designated as ciguaterin. It has been confirmed to be distributed widely in the liver and occasionally in the muscle of representative ciguatoxic fishes. This toxin, however, may be a less important factor than ciguatoxin in the development of ciguatera.

The skin of a sea bass (*Pogonoperca punctata*) has been found to contain a toxin, inducing in cats ciguatera-like syndromes when force-fed or injected. This revealed both ichthyotoxic and hemolytic activities with a closer similarity to the ichthyotoxin found in soapfish rather than to the pahutoxin in boxfish.

A water-soluble substance toxic to mice was detected in the viscera and digestive tract contents of a filefish (*Alutera scripta*). From its chemical properties this could be a new marine toxin. The toxin in the viscera was indistinguishable in chemical and pharmacological properties from that in the ingested materials, which mainly consisted of broken reef corals tentatively identified as *Goniopora* sp. This may strongly suggest the presence of toxic reef corals which serve as a primary source of toxin in the filefish and perhaps in many other coral feeders.

Further toxins have been isolated from the liver and flesh of *Gymnothorax javanicus* and from toxic crabs.

6.2 Liver toxin from *Gymnothorax javanicus*

Although it has been known for some time that livers of ciguaterix fish contain a higher concentration of toxin than does the flesh, early attempts at extraction of toxic shark livers were unsatisfactory. A procedure was devised by which toxin from the livers of the moray eel *Gymnothorax javanicus* could be extracted and purified.

In the course of this work it was discovered that toxin could be extracted not only from livers of eels with a mangoose rating of +1 to +5, but also from Johnston Island *G. javanicus* with a zero (i.e. non-toxic) mongoose rating. In fact, livers obtained from eels with mongoose ratings of 0 to +3 contain approximately the same amount of toxin per unit weight of liver. Livers from *G. javanicus* (Johnston) with mongoose ratings of +4 and +5 possess distinctly larger amounts of toxin per unit weight of liver.

By a series of solvent extractions, column and thin layer chromatographies on various adsorbents in different media, an homogeneous toxin of ca. 1.6 ppm yield and an LD_{100} of 0.03 mg/kg was obtained. To date, a total of 8 mg of this toxin extracted from 5.1 kg of eel livers has been produced.

The intact toxin is not sufficiently volatile for mass spectrometry and its infrared and nuclear magnetic resonance spectra are uninformative. Hydrolysis of the toxin under acidic conditions yields, in contrast to similar treatment of ciguatoxin, no glycerol or related small molecules and only 3% by weight of fatty acid residues. The nitrogenous moiety is unstable but bears in its mass spectral characteristics considerable resemblance to the nitrogenous moiety of ciguatoxin. Both degradative fragments exhibit a mass peak of 355 mass units, which by high resolution mass measurement has a composition of $C_{20}H_{35}N_3O_3$.

It may be possible to demonstrate that the nitrogenous portions of ciguatoxin and of the liver toxin are identical or closely related. Furthermore this nitrogenous fragment is perhaps the common denominator of all molecular entities which are capable of producing ciguatera symptoms or characteristic pharmacological reactions.

6.3 Ciguatoxin

Ciguatoxin as isolated from the flesh of *Gymnothorax javanicus* is a yellowish oil which is homogeneous by the criteria of thin-layer chromatography. The toxicity (minimum lethal dose) of this homogeneous product ranges from 0.1 - 0.5 mg/kg (by intraperitoneal injection in mice). The toxic principle is unstable and considerable losses of activity occur during purifications even when carried out at low temperatures (5 - 7° C).

Chemical and spectral data suggest the presence of several amine moieties. Chemical degradation reveals the presence of glycerol, fatty acids, long chain hydrocarbon, water-soluble and organic-soluble amines.

Chemical and physical data thus indicate that ciguatoxin appears to be a large and highly complex molecule containing lipid, hydrocarbon, and several amine moieties.

6.4 Toxin from crabs

Certain toxic crabs found at scattered locations in the Pacific have been studied to determine if they are possibly related to ciguatera fish poisoning. These toxic crabs are well known by people in the Ryukyu and Amami Islands where illness and death among humans, pigs and chickens have occurred.

Three species of xanthid crabs have been implicated: *Zosimus aeneus*, *Platypodia granulosa* and *Atergatis floridus*.

The toxin was found to be soluble in water and methanol but insoluble in most fat solvents. Methods of purification were those used for paralytic shellfish poison. The purified toxin was found to be indistinguishable from saxitoxin but different from tetrodotoxin. No relationship to ciguatera fish poisoning was evident.

6.5 Pharmacological action

Ciguatoxin extracts from the flesh and liver of moray eels (Gymnothorax javanicus) have been tested by intravenous injection in rats. To show the pharmacologic action, respiratory rate, pulse rate and blood pressure tracings were recorded continously before and after injection. In the otherwise untreated animal, ciguatoxin produced transient apnoea, hypotension and bradycardia immediately following or during administration, followed shortly by re-establishment of respiration with hypertension and tachycardia. To determine the physiological origin of these reactions, rats were subjected to several procedures such as resection of the ninth and tenth cranial nerves and medication with drugs of known mechanisms of action.

The results of these investigations indicate direct muscarinic and nicotinic cholinomimetic effects which are not the result of anti-cholinesterase action. Atropine has been found to be an antagonist of the peripheral muscarinic actions although it is not effective against the central respiratory failure produced by ciguatoxin; in large dosage, some protection against peripheral nicotinic actions has also been observed. Comparison of toxins at different stages of purification has shown that there is some reduction in the number of cholinergic sites affected by the most purified toxins. On the other hand the pure liver toxin appears to be pharmacologically identical to one of the purified flesh fractions. It is concluded that the crude toxin may contain more than one toxic molecule but that all toxin types have a basically similar "cholinergic" mechanism of action.

7. Bio-assays

Field studies on ciguateric fish have been limited by the lack of a simple text for toxicity. Lack of knowledge of the chemistry of the toxin together with the multiplicity of toxins have not permitted the development of any chemical tests. The only practical tests applicable have been bio-assays using cats, rats, mice, mongooses and chickens. Both the intra-peritoneal injection of extracts into mice and the mongoose feeding test have been utilised fairly widely as bio-assays but both have their limitations and difficulties.

A new test has been developed utilizing the stream crayfish of the southern United States (Procambarus clarkii, Girard) and an emulsified homogenate of the liver. The crayfish was chosen as it has twice been reported to be sensitive to toxic fish; extract of fish liver was chosen as it may contain up to 50 times as much toxin per unit weight as flesh.

In the testing technique fish liver is minced and then homogenized mechanically in a solution of 4.5% Tween 60 (Polyoxethelene sorbitan monosterate) in 0.9% NaCl, at the ratio of 1 g liver to 2 ml solution. This homogenate is heated to 60° C for 30 minutes and then centrifuged. The resulting supernatant fluid is used to inject crayfish at 0.33% body weight on the ventral side of the second abdominal segment, lateral to the midline.

If the crayfish is injected with a blank control solution, or with extract from non-toxic fish, there is no reaction. If the crayfish is injected with extract from a toxic fish, it reacts in one of the following ways: transient convulsions with abdominal flexure, loss of aggressive posture with the chela, awkward stance and twitching of appendages, loss of righting reflex or moribundity and death. Of these reactions, the loss of righting reflex is the most convenient for purposes of assessment. This loss of righting reflex is defined as existing when a crayfish which has turned over, or has been turned over on to its back with a rod fails to right itself after two minutes.

Statistical tests have shown that the average time taken for the "loss of righting reflex" to develop in five crayfish after injection of liver extract will differentiate between moderately and highly toxic fish. Reasonable correlation is found with the results of the mongoose test, and initial evidence indicates a correlation with the amount of toxin recoverable from the flesh.

The test was developed using toxic moray eels, Gymnothorax javanicus from Johnston Island. Ten other species of fish from other islands also showed correlations with the mongoose test. In Ctenochaetus striatus, the "maito" from Tahiti, the reaction was so immediate and violent that it may have been caused by another toxin, the presence of which has been indicated by previous studies.

As the crayfish is not available in most archipelagoes, and as the stream shrimp, Macrobrachium, is found on most high islands, the results with the crayfish were compared to the reactions induced in M. rosenbergi (a Malaysian species introduced to Hawaii). Here, in eight tests using eels, "maito" and red snapper, there is a reaction qualitatively, and probably quantitatively, similar to that of the crayfish.

8. Origin of the toxin

The mechanism by which fish become toxic is not known but several theories have been propounded as the result of some consistent observations in the development of the phenomenon. It is certain that there is no simple explanation and the causation is considered to be complex. It has been shown that toxicity can be induced by diet and also that this toxicity is retained with little or no loss. The presence of a toxic food item in one area and its absence in another offers a reasonable explanation for the varying distribution of ciguatera. Further evidence of the possible importance of diet is given by the fact that larger individuals within a species of fish have higher toxicity through longer opportunity to consume toxic material. Recent observations have singled out algae as the prime suspect organism in the production of toxins of ciguatera or, at least, of its precursor substances. There is some justification for such reasoning particularly since some obligatory herbiverous fish such as surgeon fishes and parrot fishes may cause ciguatera when eaten. It is possible that some other organism or organisms are ingested incidentally with benthic algae. Such organisms could be sponges, fungi or bacteria growing in close proximity to algae.

While algae or organisms closely associated with algae present an acceptable hypothesis of origin of the toxin, it has been observed that fish develop toxicity in areas where there has been creation of new submarine surfaces as the result of shipwrecks, aircraft wrecks, dumping of war materials, dredging, construction or natural substrata disturbance. It is possible that such exposure presents suitable algal settling areas under certain conditions. This is substantiated by experimental work in which algal growth on newly exposed submarine surfaces such as plywood has been examined.

8.1 The rôle of algae

Algae, especially those which grow early on new surfaces, have been collected from reefs where poisonous fishes are found in the Society Islands for testing of toxicity, but none displayed any significant quantity of toxin. The first alga tested was the chrysophyte Chrysonephos lewisii; it was clearly the dominant organism on surfaces of concrete, coral rock, and durotex placed on three toxic reefs in Tahiti and one in Moorea during the southern winter after a period of two to three weeks.

The green alga Enteromorpha sp. and the brown Ectocarpus sp. succeeded Chrysonephos on the new surfaces after about a month in the sea. Still later these algae were largely replaced by many species such as the bluegreens Calothrix crustacea and Lyngbya majuscula, the brown Sphacetaria sp., the green Cladophora sp., and the reds Polysiphonia sp., Centroceras clavatum, and Laurencia sp. These algae were tested en masse. The bluegreens Hormothamnion enteromorphoides, Lyngbya aestuarii, and Calothrix sp. and assorted diatoms were the principal algae growing on a concrete surface placed on a sand bottom in the lagoon near a toxic reef in Tahiti; this surface was subject to some silting by sand. A new surface placed in the sea on December 23 for twenty days yielded primarily Cladophora, and one in 15 days beginning February 29 mainly Enteromorpha (with some Eryptotrichia, Sphacelaria, and Cladophora). The bluegreen Phormidium sp., which was found growing naturally at the edge of Teavaraa Pass, Tahiti, was also tested. The two mice injected with extracts of this alga died in 34 minutes at the dosage of 2000 γ/g. Deaths from other algae required at least 3000 γ/g. A test with seawater alone produced death in two mice in 85 minutes at 3500 γ/g.

The following algae were collected at toxic sectors at Ishigaki in the Ryukyu Islands: the green Chlorodesmis sp., the diatom Synedra sp., and the bluegreens Phormidium sp. and Lyngbya sp. None were toxic beyond the level of 3000 γ/g.

Although these results do not suggest that any of the lagae tested are the source of ciguatera toxins, it should be remembered that the toxic level in the responsible organism may be very low and thus difficult to demonstrate by direct feeding or injection of extracts into mice. Also it is possible that a precursor of the toxic substance occurs in an alga which exhibits no toxic properties until it is metabolized by animals.

8.2 The rôle of other organisms

A limited number of observations on the stomach contents of fish have shown the presence of two types of yeasts in toxic fish but only one in non-toxic fish. Insufficient work has been performed to draw any conclusions from this and further investigations are required before the possible rôle of yeast as a toxin producer can be elucidated.

8.3 The rôle of newly exposed underwater surfaces

Disturbance of submarine substrata as the result of blasting, underwater construction, ship or aircraft wreck, disposal of war material, etc. have been frequently recorded as being followed by the appearance of toxicity in fish. An example of this effect was reported from the island of Hikueru in the Tuamotu Group. Here a wharf was constructed using reef material but its site was unsuitable for ships working at certain states to tide and sea so that unloading then was necessary at a point two miles distant. Within two years, fish in both areas became toxic and the incidence of toxicity subsequently extended until they fused. Another incident demonstrates equally this effect. Two aircraft crashed into a lagoon. In view of the possibility of development of toxicity in fish, attempts to remove both wrecks were made, but only one was successfully removed. The other remained submerged and, about it, fish developed toxicity. It would appear from such observations that the exposure of new submarine surfaces as well as disturbance of substrata does result in the appearance of toxicity.

The relationships of exposure of new submarine surfaces to the appearance of subsequent fish toxicity has been the subject of investigation and this has been directed towards examination of marine growths, particularly algae, which appear on these surfaces. In water which is clear, without heavy surf, which is warm and which is not too deep, algae and a slime consisting of diatoms, bacteria and other organisms develop rapidly and fish do feed on such material.

It is possible that newly exposed surfaces not only provide suitable algal growth areas but also present local chemical changes of water content, in turn absorbed by algae. Such absorption with subsequent concentration or metabolism may be one of the factors predisposing to the eventual production of toxin.

8.4 The rôle of pathological conditions in fish

Another theory on the possible origin of toxicity in some fishes was developed when cases of severe osteitis lesions, especially marked on the skull, were found among populations of toxic fishes. Those might be caused by parasitic isopodous hematophage crustucea entering through the gills and nostrils, which might be the vectors of microbial infection. This hypothesis is based on the fact that, in some areas of the Caribbean, toxic fishes are only found in well-defined places. Supporters of this theory think that the fishes congregate in the same place because, being ill and tired, they seek a quiet place or a shelter, for instance among wreckage.

8.5 Investigations into origins

Few planned studies into ecological aspects of fish poisoning have been undertaken but one such is reported from Tahiti. This was in relation to the surgeon fish, *Ctenochaetus striatus*, known as "maito", was undertaken because it is the most frequently implicated species in ciguatera outbreaks in French Polynesia. The studies have been centred about the Atimono region and have been directed at the habits, migrations and feeding patterns of the species in relation to toxicity. So far, the results are only preliminary but it appears that the "maito" migrate off the edge of the reef diurnally and the same individuals can be found in the same area nearly one year later. Material ingested by the fish consists of fine algae, organic detritus and sand grains. More than 50% of individual specimens caught and fed to small cats proved to be toxic. The occurrence of toxic fish was found to a water depth of at least 20 metres. Contrary to local opinion, no non-toxic zones were found and,as most distribution data to date on fish poisoning has been based on such local opinion, this finding is significant in emphasising the need for scientific investigation into the problem.

9. Medical treatment

The treatment of ciguatera will remain of primary significance as long as no scientific method has been developed to prevent fish poisoning and,in many cases, traditional medications still compete with modern techniques.

9.1 Symptomatic treatment

Until the present time, the treatment of ciguatera has been purely the administration of appropriate drugs to relieve symptoms.

Vitamin B complex, colchicine and salicilic acid have been used to reduce neurological disorders; synthetic antihistamines and magnesium hyposulphite for pruritis; antispasmodic drugs, lactic acid bacilli and vegetable charcoal for digestive tract disorders; and analeptics with steroids for cardiovascular shock.

Where dehydration is present, fluid and electrolyte replacement therapy plays an essential part in the initial phase of severe toxicity.

9.2 Specific treatment

9.2.1 Oximes

As the result of recent investigations, oxime-type cholinesterase regenerators have been tried both alone and in association with atropine. Their action appears reasonably effective in relieving the vagal hyper-exitation symptoms of ciguatera, especially when administered early.

9.2.2 Ion chelating agents

Chelating agents act by combining with heavy metallic ions to form stable complexes in which metal is dissimulated, thus losing its toxicity. The structure of the ciguatoxin molecule appears to simulate a heavy metal in this type of action and chelating agents have had some success.

Both oximes and chelating agents require to be administered in hospital at an early stage and their use is thus necessarily limited.

10. Ichthyosarcotoxism and marine protein food resources

10.1 The increasing world population pressures, the urgent need for establishing a solid fishing economy among under-developed countries, the need for low-priced high quality protein food products by almost two-thirds of the world's population, and the rapid advance and expansion of insular shore fisheries all indicate the importance of establishing a better understanding of the marine protein food potential.

The oceans annually produce about 400 million metric tons of animal protein in a variety of species and sizes suitable for harvest and use by man. Since 1850, the world catch of fishes and shellfishes has progressively increased from about 1.5 million tons to about 50 million tons in 1964, less than ten percent of the total catch coming from fresh water. Analysis of the total catch reveals that about 85.9 percent of the organisms were fishes, and the remainder were shellfish (7.6 percent), whales (5.1 percent), and other aquatic animals (1.4 percent).

Despite the fact that ichthyologists recognize about 25,000 species of fishes, less than 13 kinds make up the bulk of the world's fisheries catches. A few kinds of fishes are heavily exploited, some are fished but unexploited, and others are abundant but go unharvested. Recent studies indicate that the rapidly growing fisheries are not in northern or southern latitudes but rather are in tropical seas and it is anticipated that this trend will continue with even greater efforts being directed to tropical insular areas.

The old perspective of viewing the ocean as a source of food in the direct form of fish (fresh, frozen, dried or canned) is rapidly expanding to include fish meal, fish protein concentrates, and other protein derivatives. In addition, various other forms of marine organisms, i.e. marine invertebrates and plants, are also being contemplated as potential sources of marine protein concentrates. The use of fish protein concentrates (FPC) from low-priced marine fishes has been strongly recommended by most food economists as one of six possible solutions to the problem of the world protein deficit. FPC is particularly meritorious because it is a high quality protein; it can be cheaply produced; untapped fish resources are abundantly available in many parts of the world. The material can be easily stored and transported with minimal problems.

With the immediate prospects of developing a broad spectrum of food organisms ("trash species") from subtropical and tropical oceans, we are now faced with many serious biotoxicological food problems which demand immediate attention on a circumtropical basis.

Information concerning the extent and precise distribution of ichthyosarcotoxism in fishes is limited at the present time, thus requirements for public health surveillance and for the development of commercial fishing operations present considerable difficulty. Efforts to develop protein concentrates from subtropical and tropical marine fishes pose some very serious questions concerning their safety, as they may be produced from fishes containing ichthyosarcotoxins or industrially-produced toxic compounds.

10.2 Ichthyosarcotoxism acts as a limiting factor in current economic growth, greatly hampering the production and sale of coastal fish, as exemplified in French Polynesia. By its existence, both fishermen and consumers avoid toxic or reputedly toxic fishes, thus leading to possible upset of marine faunal ecology with over-fishing of "safe" species and proliferation of the others. In turn this could result eventually in further increase in the incidence of fish poisoning.

The crucial issue is how to control production in such a way that it can develop without endangering the health of the consumer and without creating proliferation of toxic species. With many unknowns, the drafting of even limited legislation is virtually impracticable and unlikely to afford real protection in any case. Another alternative could be the utilisation of lagoons which, being enclosed areas, appear to be ideally suited to various forms of marine agriculture and which play an increasingly prominent part in the development of ocean resources. If it is considered likely that the origin of toxicity is to be found in the environment, the question arises as to what requirements must be imposed on future breeding areas in order to protect the consumer against possible toxicity as the result of production.

To utilise marine resources in an attempt to make them available for reduction of protein food deficiency in a safe manner requires considerably more knowledge that is at present available. There is a great and urgent need for expansion and coordination of research to prevent ecological disturbance, with its attendant problems, and to permit the safe utilisation of marine protein.

RECOMMENDATIONS

1. The Seminar recommends that particular attention be devoted to the epidemiological and ecological aspects of marine biotoxications (especially ichthyosarcotoxism). This should include the documentation, collation, identification of causative agents, biology, clinical data, incidence of outbreaks, and economic implications. All these data should be coordinated, collected, and distributed by an international agency.

2. The Seminar recommends that the South Pacific Commission requests the World Health Organization that ichthyosarcotoxism and marine biotoxication be included amongst the reportable diseases, since these represent:

(a) an important, world-wide, public health problem on which little is known; and

(b) serious impairment of the future development of world food resources of marine origin.

3. The Seminar recommends that intensive study be given to the problems of marine biotoxications since they are a limiting factor in the future economic development of marine aquaculture techniques.

4. The Seminar recommends that the various governments grant every financial and administrative support to any international research programme on marine biotoxication and especially ichthyosarcotoxism that might be established in their territory.

5. The Seminar recommends that intensive study be given to the development of a more suitable bio-assay method for use in field investigations on marine biotoxication.

6. The Seminar recommends that periodic meetings be held to exchange information on all aspects of marine toxins and other marine substances with useful biological activity. These meetings should be held approximately every two years starting with participation in the 1970 Twelfth Pacific Science Congress at Canberra, Australia.

7. The Seminar recommends that the South Pacific Commission publish a regular newsletter at about 3 to 6 monthly intervals providing information on fish poisoning and research activity to public health workers and interested workers.

8. The Seminar supports the proposal of the Technical Meeting on Fisheries held in Noumea in June 1968 for the establishment of an agency specifically charged with responsibility for initiating and assisting the rational development of the resources of the reef and lagoon and immediate offshore waters, and urges that the Agency support research on ichthyosarcotoxism.

9. The Seminar recommends that the future economic potential of marine biochemical agents be explored as to their use as new foods and pharmaceutical products.

10. In view of reliable information on serious and continuing outbreaks of fish poisoning in the Marquesas Islands, and in view of the potential information that can be gained from a study of such a highly toxic area, the Seminar recommends that a coordinated scientific study be undertaken employing those specialists that can most profitably exploit this situation. It further recommends that this endeavour make a special effort to obtain toxic raw material needed for later chemical and pharmacological studies.

ANNEX I

A G E N D A

16th August 1968

9.00 a.m.	Official Opening Briefing on Seminar Procedure Election of officials
Afternoon	Field visit to the Marine Biology Laboratory at Papara.

18th August 1968

All day	Trip to Rangiroa (Tuamotu Islands) - Visit to Fisheries Department facilities at Avaetoru (cold storage, fish pond). - Exhibition of commercial and toxic fish species by J. Tapu, World Champion of under-water spear-fishing. - Description of work being done on sharks by T.W. Brown, of the British Overseas Deep Sea Diving & Salvage Service, Australian Deep Sea Diving and Salvage Service.

19th August 1968

Morning	Actual significance of fish poisoning in general, and particularly of ciguatera, in the various participating territories. Geographic distribution. Public health aspects. Development of ichthyo-sarcotoxism.
Afternoon	Epidemiological aspects: major ciguatoxic species, apparent seasonal variations. Clinical aspects: variations according to species; the sensitization and summation concepts. Paraclinical aspects: emphasis in complementary tests.

20th August 1968

Morning	Present knowledge of marine toxins which may be a cause of fish poisoning. Pharmacological and chemical characteristics of the various toxic fractions.
Afternoon	Problems of experimenting with animals; various techniques, in particular, the possible use of invertebrates. Early diagnosis of fish toxicity: the value of empirical notions, advances in the development of a scientific technique. Experimental induction of toxicity in non-toxic fishes.

21st August 1968

Morning	Present knowledge regarding the biological origin of toxicity. Corals, sea-weeds, plankton, bacteria, toxic marine fungus. Study of the various food chains. Possible incidence on the production of ichthyotoxins. Ecological study of some ciguatoxic species. The ciguatoxic invertebrates.
Afternoon	Factors promoting the genesis of ichthyosarcotoxism. The rôle of some metallic salts, pollution by the soil, new surfaces. Value of algae, plankton or bacteria cultures. Treatment aspects: the rôle of chelating agents, oximes, B-vitamin complexes, especially B6, and antihistamines.

22nd August 1968

Morning	The significance of marine biotoxins. Implications of ciguatera for the development of lagoon animal and plant resources. Ciguatera research in a programme of fisheries research.
Afternoon	Closing Session : General Report Recommendations

ANNEX II

LIST OF PARTICIPANTS

T. ABE	Principal Research Officer Tokai Regional Fisheries Research Laboratory 5, 5-chome, Kachidoki Chuo-ku TOKYO. JAPAN.
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C. LE BIGOT	Chef des Services des Affaires Maritimes et de la Pêche PAPEETE. TAHITI.
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SECRETARIAT

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S. EXBROYAT (Mlle)	Conference Officer South Pacific Commission NOUMEA. NEW CALEDONIA. Secretary to the Seminar
M. NOELLAT (Mme)	Stenographer South Pacific Commission NOUMEA. NEW CALEDONIA.
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ANNEX III

LIST OF WORKING PAPERS

SPC/ICHT/WP. 1 - Recent cases of ichthyosarcotoxism recorded in British Solomon Islands Protectorate.

SPC/ICHT/WP. 2 - Clinical forms of fish poisoning of the "Ciguatera" type in French Polynesia, by Raymond Bagnis.

SPC/ICHT/WP. 3 - Ciguatera in Tahiti: An endemic disease, by R. Bagnis, J. Bennett, V. Joutain, and F. Nanai.

* SPC/ICHT/WP. 4 - The significance of marine biotoxins to marine protein concentrates and food from the sea, by Dr Bruce W. Halstead.

SPC/ICHT/WP. 5 - Occurrence of toxic crabs in the Ryukyu and Amami Islands and similarity of the crab toxin to Saxitoxin, by Shoji Konosu and Yoshiro Hashimoto .

SPC/ICHT/WP. 6 - Toxins found in ciguatoxic fishes in the Ryukyu Islands, by Yoshiro Hashimoto.

SPC/ICHT/WP. 7 - Ciguatera in the Ryukyu and Amami Islands, by Yoshiro Hashimoto, Shoji Konosu and Takeshi Yasumoto.

SPC/ICHT/WP. 8 - Somes cases of ciguatera and cognate ichthyosarcotoxism reported from Tokyo, by Tokiharu Abe.

SPC/ICHT/WP. 9 - Outline of ichthyosarcotoxism in New Caledonia, by G. Chemier

SPC/ICHT/WP.10 - Ichthyosarcotoxism (Fish Poisoning) in New Guinea, by A.M. Rapson.

SPC/ICHT/WP.11 - Comments on some cases of ciguatera observed in American Samoa, by Ernest Thompson.

SPC/ICHT/WP.12 - Selected list of bibliographical references on ichthyosarcotoxism.

SPC/ICHT/WP.13 - The biology of Ctenochaetus striatus, a known ciguateric acanthurid fish of Tahiti, by Charles K. Walters.

SPC/ICHT/WP.14 - Ciguatera in Queensland, by G.D. Broadbent.

* SPC/ICHT/WP.15 - Cerebellar syndrome in Ciguatera, Notes on two cases, by M. Barbotin and R. Bagnis.

* SPC/ICHT/WP.16 - Pharmacological reaction of the rat to ciguatoxin, by Martin D. Rayner.

* SPC/ICHT/WP.17 - Liver toxins from Gymnothorax javanicus, by Takeshi Yasumoto and Paul J. Scheuer.

* SPC/ICHT/WP.18 - A new field and screening bioassay for ciguatera, by Emile Keene, Helen A. Randall and Albert H. Banner.

* SPC/ICHT/WP.19 - Ciguatoxin, by Wataru Takahashi and Paul J. Scheuer.

* SPC/ICHT/WP.20 - Marine algae as a possible source of ciguatera toxins, by John E. Randall.

* SPC/ICHT/WP.21 - Patterns of development of ciguatera fish poisoning in the Pacific, by Philip Helfrich.

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* Abstract only

* SPC/ICHT/WP.22 - Ciguatera-type food poisoning on the island of Reunion (1966-67) and J. Morice's theory regarding the origin of the toxicity of some tropical fish species, by Alain Lebeau.

SPC/ICHT/WP.23 - Notes on the use of oximes in the treatment of ciguatera, by R. Bagnis

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SPC/ICHT/WP.1
5th April 1968

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August 1968)

RECENT CASES OF ICHTHYOSARCOTOXISM RECORDED IN BRITISH SOLOMON ISLANDS PROTECTORATE

Central District : (Guadalcanal, Savo, Nggela, Russell Islands, Rennel, Bellona, Santa Ysabel)

No cases said to be on record.

Eastern District : (San Cristobal, Ulawa, Santa Cruz, Ugi, Santa Ana, Vanikoro, etc.)

Red snapper: reported to be 2 varieties around Solomons. One can cause an illness characterised by vomiting, muscular aches and pains (Santa Cruz), no fatalities reported.

From different sources (Ulawa and Ugi) reported that this fish is very toxic to infants - several cases reported of babies having convulsions similar to tetanus - after mother ate supper. Toxin presumably ingested in breast milk.

Grouper: also reported to be toxic to infants of lactating mothers. No cases of fatal poisoning reported.

Barracuda: intolerance to barracuda reported by the Ariki Kafika (Tikopia) who reports that he had an illness consistent with ciguatera poisoning after eating this fish. Vomiting and nausea were followed by muscular pains and weakness lasting several days. Other Tikopians similarly affected.

Puffer fish: cases previously encountered in the Solomons of tetraodon poisoning which, on occasion, has been fatal - 1958, two deaths recorded in Honiara (from Rove) within a few hours of ingesting sharp-backed puffer fish, also 1962, death in Reef Islands from the same fish. Smooth puffer and sharp-backed puffer recognised as poisonous fish in this District and avoided. Spiny puffer only eaten after thorough cleaning and removal of gonads.

Trigger fish, filefish and boxfish: are commonly eaten, although there has been one case of poisoning from boxfish (Dr Beck).

Moray eel (*Gymnothorax flavimarginatus*) : considered poisonous in Tikopia and avoided, but eaten in Santa Cruz and Cristobal. Toxicity encountered in Santa Cruz.

Turtle: some time ago (?1963), two people died, one adult, one child, in Santa Ana after eating turtle. One other man who ate some of the same turtle was admitted to Kira Kira Hospital seriously ill- but survived.

Western district : (New Georgia, Choiseul, Vella Lavella, Shortland Islands, etc.)

By eating:

Rino - (Roviana) Rennel and Bellona, worse at Bellona. (The latter two being in Central District).

Turtle, Hawk's Bill - occurred about three times on Malaita and once on Santa Ana. One fatality in the former case.

Puffer fish - fine and short spikes, poisonous.

Mihu - (Roviana) rare occasions (Western District)

Buma - only when it eats jelly fish and if eaten with guts and gills (some natives do). Buma is a small sardine-like fish.

Original text: English

SPC/ICHT/WP.2
16th April, 1968.

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

SUMMARY

CLINICAL FORMS OF FISH POISONING OF THE "CIGUATERA" TYPE IN FRENCH POLYNESIA

by Raymond BAGNIS
Naval Captain, Medical Officer

Fish toxicity has existed in French Polynesia for a very long time, the majority of cases being of the ciguatera type.

The results of 350 clinical observations recorded over a three-year period have been analysed: detailed description of the complete form with - basically - its four characteristic syndromes: digestive, cardiovascular, neurological, algic, and an associated syndrome the components of which are more or less constant; the course normally followed leads towards recovery without after-effects within a period ranging from three days to one month.

The type of description chosen for the purpose of the exposé is the most characteristic, but a certain number of factors come into play which multiply the number of particular cases. Consequently the author has endeavoured to establish a classification of the clinical forms, bearing in mind the polymorphism of the symptoms, the sensitization phenomenons and the rôle of the causal species.

There follows a consideration of the problem of ciguatera diagnosis; this is determined on the basis of three specific symptoms: modification of sensitivity (permanent symptom), bradycardia and mydriasis which appear in the hours following the ingestion of a suspect fish; this clinical association enables ciguatera to be differentiated from the other varieties of fish toxicity encountered in French Polynesia.

Finally, passing rapidly over a pathogeny as yet little known, the author shows that the oximes, cholinesterases regenerators, successfully used in association with large doses of atropine and a vitamin B cocktail in the treatment of severe and complete forms of toxicity, do not appear to constitute the specific treatment eagerly anticipated during the last few years.

Work accomplished in the Medical Oceanography Section of the Louis Malardé Research Institute, in collaboration with J. Bennett, V. Joutain and F. Nanai,
Papeete, 3rd April, 1968.

SPC/ICHT/WP.3
16th April 1968

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

CIGUATERA IN TAHITI: AN ENDEMIC DISEASE

S U M M A R Y

A survey was carried out with a view to ascertaining the true extent of the incidence of poisoning by toxic fish in Tahiti, and its consequences. It covered an actual population of 33,085 persons - i.e. close on 89% of the population of the districts and the two townships of Tahiti.

The year 1966, from January to December inclusive, was chosen as the period of time in which the survey of cases of intoxication was to be made. The incidence of the illness - i.e. the total number of cases which had occurred during that period - was 2,798. The rate of incidence is 8.45%. It can be said, therefore, that this endemic disease is extensive.

This survey has also enabled a count to be made of over 40 species of toxic fish, the majority being carnivorous. This list does not include the species found in the other islands or island groups.

A survey was carried out in respect of the toxic areas and a map drawn up showing the extent of ichthytoxism in Tahiti. This map will serve as a control instrument in the event of possible changes in the toxic areas and will be placed at the disposal of anyone who may wish to consult it.

Finally, the analysis of the data collected reveals that ichthyotoxism also constitutes a fairly considerable problem in the economic field.

SPC/ICHT/WP.4
16th April, 1968

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

THE SIGNIFICANCE OF MARINE BIOTOXINS TO MARINE PROTEIN CONCENTRATES AND FOOD FROM THE SEA

by Dr. Bruce W. Halstead *

Biotoxicity and Marine Protein Food Resources: The increasing world population pressures, the urgent need for establishing a solid fishing economy among underdeveloped countries, the dire need for low-priced high quality protein food products by almost two-thirds of the world's population, and the rapid advance and expansion of insular shore fisheries all point up the drastic need to establish a better understanding of the edibility of our marine protein food potential.

The oceans annually produce about 400 million metric tons of animal protein in a variety of species and sizes suitable for harvest and use by man. Since 1850, the world catch of fishes and shellfishes has progressively increased from about 1.5 million tons to about 50 million tons in 1964. Less than ten percent of the total catch came from fresh water. The remainder were marine organisms. Analysis of the total catch reveals that about 85.9 percent of the organisms were fishes, and the remainder were shellfish (7.6 percent), whales (5.1 percent), and other aquatic animals (1.4 percent).

Despite the fact that ichthyologists recognize about 25,000 species of fishes, less than 13 kinds make up the bulk of the world's fisheries catches. A few kinds of fishes are heavily exploited, some are fished but unexploited, and others are abundant but go unharvested (Schaefer and Revelle, 1959).

Recent studies indicate that the rapidly growing fisheries are not in northern or southern latitudes but rather are in tropical seas (Chapman, 1965). It is anticipated that this trend will continue with even greater efforts being directed to tropical insular areas.

The old perspective of viewing the ocean as a source of food in the direct form of fish (fresh, frozen, dried or canned) is rapidly expanding to include fish meal, fish protein concentrates, and other protein derivatives. In addition, various other forms of marine organisms, i.e., marine invertebrates and plants, are also being contemplated as potential sources of marine protein concentrates. The use of fish protein concentrates (FPC) from low-priced marine fishes has been strongly recommended by most food economists as one of six possible solutions to the problem of the world protein deficit. FPC is particularly meritorious because it is a high quality protein; it can be cheaply produced; untapped fish resources are abundantly available in many parts of the world; FPC can be easily stored and transported with minimal problems. In general, modern technology is looking to the sea with eager anticipation as to the possible utilization of a broad spectrum of marine animals and plants as new sources of food, pharmaceuticals and biochemical agents having medical, industrial, scientific and military applications.

* Director, World Life Research Institute,
Colton, California, U.S.A.

With the immediate prospects of developing a broad spectrum of food organisms ("trash species") from subtropical and tropical oceans, we are now faced with a barrage of complex and serious biotoxicological food problems which demand immediate attention on a circumtropical basis.

We are almost totally lacking in information concerning the abundance and precise geographical distribution of ichthyosarcotoxic fishes in the tropical Atlantic, Mediterranean, Caribbean, and Indo-Pacific regions. Existing knowledge is exceedingly crude and almost worthless for the requirements of public health surveillance and the commercial development of insular shore fisheries operations. The ecological factors contributing to the biogenesis of these toxic fish products are unknown. Crinotoxic substances may serve as regulatory mechanisms governing the size and composition of populations of fishes, and may therefore be an important factor in future investigations in fisheries dynamics and aquiculture. Efforts to develop protein concentrates from subtropical and tropical marine fishes pose some very serious questions concerning their edibility, if these concentrates were to be produced from fishes containing ichthyosarcotoxins or industrially-produced toxic compounds which may be present in marine fishes.

SPC/ICHT/WP.5
13th May, 1968

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

OCCURRENCE OF TOXIC CRABS IN THE RYUKYU AND AMAMI ISLANDS AND SIMILARITY OF THE CRAB TOXIN TO SAXITOXIN

by Shoji Konosu and Yoshiro Hashimoto *

Nineteen cases of illness associated with the ingestion of toxic crabs were traced in the Ryukyu and Amami Islands. The signs were characterized by vomiting, paralysis, and rapid death. In the screening test for toxic crabs, three species, Zosimus aeneus, Platypodia granulosa and Atergatis floridus, were found to contain a paralytic toxin, which killed mice in a few minutes. Specimens of A. floridus caught along the southern coast of the main land of Japan were also poisonous.

A marked individual variation of toxicity was recognized in each species. In general, the appendages were more toxic than the cephalothorax, and the exoskeleton also contained a considerable amount of toxin. The toxin was easily dialyzable, soluble in water and methanol and insoluble in most fat solvents.

For the purification of crab toxin, the ion-exchange column chromatographic method for saxitoxin was found to be applicable without any modification, and a partially purified preparation of toxin having a toxicity as high as 1,760 M.U./mg solid (the minimum lethal dose for mice, 0.028 y/g body weight) was obtained from the water extracts of Z. aeneus.

In paper and thin layer chromatographic behaviors and the dose-death time relationship in mice, the crab toxin was indistinguishable from saxitoxin and apparently different from tetrodotoxin. The crab toxin may be identical with or closely related to saxitoxin.

Original Text: English

* Laboratory of Marine Biochemistry,
Faculty of Agriculture,
The University of Tokyo.

SPC/ICHT/WP. 6
15th May, 1968

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

TOXINS FOUND IN CIGUATOXIC FISHES IN THE RYUKYU ISLANDS

by Yoshiro HASHIMOTO *

Evidence for multiplicity of toxin involved in ciguatera has been increasingly accumulated. At least four different toxins have been demonstrated in ciguatoxic fishes in the Ryukyu Islands.

The fat-soluble toxin probably identical with ciguatoxin was found in the muscle of Lutjanus bohar and L. monostigma. This toxin may be a principal one in ciguatera, as Dr Banner suggested.

A water-soluble toxin causing transient vomiting in cats was found and designated as ciguaterin. It was confirmed to distribute widely in the liver and occasionally in the muscle of representative ciguatoxic fishes. This toxin, however, may be a less important factor than ciguatoxin for development of ciguatera.

The skin of a sea bass (Pogonoperca punctata) was found to contain a toxin inducing in cats ciguatera-like syndromes when force-fed or injected. Besides, it revealed both ichtyotoxic and hemolytic activities and a close similarity to the ichtyotoxin found in soapfish rather than pahutoxin in boxfish.

Finally, a water-soluble substance toxic to mice was detected in the viscera and digestive tract contents of a filefish (aluteres scriptus). From the chemical properties it is supposed to be a new marine toxin. The toxin in the viscera was indistinguishable in chemical and pharmacological properties from that in the ingested materials which were mainly consisted of broken reef corals tentatively identified as Goniopora sp. This may strongly suggest the presence of toxic reef corals which serve as a primary source of toxin in a filefish and presumably many other coral feeders.

Original Text : English

* Laboratory of Marine Biochemisty, Faculty of Agriculture, the University of Tokyo.

SPC/ICHT/WP.7
13th May, 1968.

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

CIGUATERA IN THE RYUKYU AND AMAMI ISLANDS

by Yoshiro Hashimoto, Shoji Konosu and Takeshi Yasumoto*

In a field investigation, the latent prevalence of ciguatera in the Ryukyu and Amami Islands was ascertained and about 20 species of fish were reported to be toxic, excluding puffers, spoiled scombroid fishes, castor oil fishes and the liver of large-sized fishes such as tunas and sharks. Among them, the following five species were found to be most important as the cause of typical ciguatera, red snapper (*Lutjanus bohar*), snapper (*L. monostigma*), moray eels (*Gymnothorax* spp.), grouper (*Epinephelus fuscoguttatus*), and sea bass (*Variola louti*). Symptoms induced by these fishes were quite similar to each other and characterized by joint ache, languor, dry-ice sensation, diarrhea and vomiting. A narrow regionality of toxic fishes was reported, and it is interesting that the areas suspected to be toxic are closely related with well-developed coral reefs.

A rabbitfish (*Siganus* sp.) was reported to induce occasionally itching, severe headache and flush of face, suggesting the presence of a different toxin. The following species were also reputed to be toxic in a few areas; *L. vaigiensis*, *L. fulviflamma*, *Plectropomus truncatus*, *Aluteres scriptus*, *Pogonoperca punctata*, *Sphyraena picuda*, *Lutjanus* sp., and *Cheilinus* sp.

Besides these fish poisonings, 4 cases due to toxic turtles, about 20 due to toxic crabs, and 5 due to coconut crabs were located respectively.

Original Text: English

* Laboratory of Marine Biochemistry,
Faculty of Agriculture,
The University of Tokyo.

SPC/ICHT/WP.8
22nd May, 1968.

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

SOME CASES OF CIGUATERA AND COGNATE ICHTHYOSARCOTOXISM REPORTED FROM TOKYO

by Tokiharu ABE*

Despite every effort to stop selling dubious fishes from alien waters, a few cases of ciguatera or cognate poisoning caused by eating fish have been observed in Tokyo and vicinity. The following three cases of fish poisoning are briefly reported here.

1) Ciguatera caused by barracuda. In May, 1949, about thirty persons of five families in Tokyo suffered from ciguatera by eating a barracuda, Sphyraena picuda Bloch & Schneider, taken by tuna long line, set somewhere in the tropical region of the Pacific Ocean. Professor Y. Hashimoto found a new type of toxin from the fillet of the fish.

2) Ciguatera caused by a lutjanid or another red fish. In July, 1966, eleven persons suffered from ciguatera by eating a red fish measuring ca. 60 cm. in total length. The fish, two in number, were given by fishermen who happened to anchor at the pier of the Central Wholesale Market of Tokyo Prefecture, to boys of a junior high school visiting the market. They asked a fish retailer to make fillets of the fish, and distributed them to their families and friends for supper. All the persons, eleven in number, who ate the fish suffered from vomiting, diarrhea and paralysis within one to seven hours after the supper.

3) Poisoning caused by eating dried flesh of a puffer. In March, 1968, two persons in Tokyo were poisoned by dried flesh of a puffer (tetraodontid), Fugu vermicularis radiatus (Abe), taken probably by trawl in East China Sea or adjoining water. Thanks to an ordinance issued by the Metropolitan Government, poisoning by fresh or frozen puffers rarely occur in Tokyo and vicinity, but dried puffers are usually sold to retailers without being passed through the Central Wholesale Market of Tokyo Prefecture. The dried flesh of puffers has long been regarded as nontoxic. The symptoms were slight.

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM

(Rangiroa, French Polynesia, 16th - 22nd August,1968)

OUTLINE OF ICHTHYOSARCOTOXISM IN NEW CALEDONIA

by

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In New Caledonia, ichthyosarcotoxism presents a problem which is both economic and medical.

In fact, the relatively high incidence of this disease in the Territory acts as a brake on the marketing of fish, despite the abundant quantities in which it is found along the New Caledonian coastline.

It is impossible to assess the extent of this disease, for in the absence of a specific therapy, and in view of the generally mild nature of this illness which can be treated at home, most patients do not consult a medical practitioner and use medication, often based on vegetable infusions, for which the "prescription" has been passed on from mouth to mouth.

For this reason, although this disease seems very widespread, no more than a dozen admissions for ciguatera are recorded annually in the Gaston Bourret Hospital of Noumea.

Most of the species of fish caught both in the lagoon and near the reefs are liable to cause this disease. Its incidence, however, seems proportional to the size of the fish ingested, the larger type being more likely to give rise to disorders than the small ones (with the exception of sardines). The species most often affected belong to the Serranidae, Lethrinidae and Scomberomoïdae families.

The symptoms shown by the patients are variable: the most common are symptoms which affect the nervous system (dysesthesia, various kinds of algesia, asthenia). In other cases, disorders of the alimentary tract (vomiting, diarrhoea) or the cardiovascular system (drop in blood-pressure) predominate.

Bearing in mind the possibility of a metallic origin, an attempt was made at the Gaston Bourret Hospital to treat ichthyosarcotoxism by an ion chelating agent, the calcic disodic salt of ethylene - diaminc - tetra - acetic acid (calcic E.D.T.A.)*

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* Patented under the name of disodic Calcitetracemate (Laroche Navarron Laboratories).

This salt which has the property of combining with heavy metallic ions to form stable complexes in which the metal is dissimulated and loses its toxicity, is used in the treatment of heavy metal intoxications (saturnism in particular).

In the treatment of ichthyosarcotoxism, this drug was administered in slow perfusions, from an ampoule containing 10 ml of E.D.T.A. in 250 ml of isotonic glucose or salt serum, at the rate of 1 or 2 perfusions per day, and this for a period of 2 to 4 days depending on the gravity of the case. In the majority of cases (13 out of 15) the treatment results in a clinical recovery. The value of this therapeutic method is limites by the need for hospitalization; nevertheless, it has a useful application in serious cases.

Original text: French

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM

(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

ICHTHYOSARCOTOXISM (FISH POISONING) IN NEW GUINEA

by A.M. Rapson *

Rossel Island has been most seriously affected by fish poisoning. In New Guinea generally, puffer fish, box fish and Grammistes caused the fatalities and chinaman fish, red bass and pike the most serious cases of illness. Although chinaman fish are poisonous in the Milne Bay district this species caught in deep water off North New Britain is sold in Rabaul. Statistics are a guide to the distribution of ichthyosarcotoxism but there is no way of judging what the real death or sickness rate may be. Cases from Rossel Island describe people suffering from fish poisoning eating the same species caught in the same locality as the fish which made them sick without any affects whatsoever. Rossel Island is surrounded by one of the biggest lagoons in New Guinea, and there appears to be little doubt that toxins are developed mainly in inshore reef or shallow waters. A small fish caught in New Ireland waters and called there talultaman is boiled wrapped in the leaves of beach lilies as soon as it is caught; otherwise it causes stomach cramps and further investigation of this use of beach lily leaves may yield information on the phenomenon of ichthyosarcotoxism. Local remedies for fish poisoning may also on investigation be found to have value in making poisonous fishes fit for food. The feeding of fishes on luminescent organism is a possible cause of toxicity. Case histories are normal and medical remedies and local cures are described. Deaths from eating shellfish and from red tide are included from an open coastal area where few fish poisoning cases are recorded, in an attempt to link biological data with a possible relationship between plankton and bottom feeders. The known cases of red tide poisoning occur off reef areas on open coasts while the principal cases of ichthyotoxism are from lagoon areas.

Original Text: English

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SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

COMMENTS ON SOME CASES OF CIGUATERA OBSERVED IN AMERICAN SAMOA

by

Dr. Ernest Thompson*

The author wishes to point out the fact that at this point there is no specific method available to the people of Samoa by which they could recognize which fish is toxic, excepting of course the unfortunate "Try-it-out" test; that the toxin is thermostable and is not water soluble; that an attack of poisoning will not impart immunity; that the toxin is not the result of bacterial action or putrefaction; that the body's adaptive and defensive processes are slow in neutralizing or eliminating the toxin; and, finally, that the seaweed AGA (Samoan term) and the toxin it bears is to be further investigated.

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia)
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COMMISSION DU PACIFIQUE SUD

SEMINAIRE SUR L'ICHTYOSARCOTOXISME
(Papeete, Polynésie Française)
16 - 22 août 1968

SELECTED LIST OF BIBLIOGRAPHICAL REFERENCES ON ICHTHYOSARCOTOXISM

This document presented by the South Pacific Commission to the participants attending the Seminar on Ichthyosarcotoxism is not a Bibliography in the strict sense but rather a list of references drawn up from our Library Index Cards, and from titles of articles which were brought to our notice. This list is certainly incomplete; we hope, however, that it will be useful to those who require information which cannot be found in the working papers prepared for the Seminar.

LISTE CHOISIE DE REFERENCES BIBLIOGRAPHIQUES SUR L'ICHTYOSARCOTOXISME

Ce document présenté par la Commission du Pacifique Sud aux participants du Séminaire sur l'Ichtyosarcotoxisme n'est pas une bibliographie au sens propre du terme mais plutôt une liste de références établie d'après les fiches existant dans notre bibliothèque et les titres d'articles portés à notre connaissance. Elle est certainement incomplète; nous espérons néanmoins qu'elle pourra être utile à ceux qui voudraient rechercher des renseignements qu'ils ne trouveraient pas dans les documents de travail préparés pour le Séminaire.

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7 August, 1968

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

THE BIOLOGY OF CTENOCHAETUS STRIATUS, A KNOWN CIGUATERIC ACANTHURID FISH OF TAHITI

by Charles K. Walters*

Ctenochaetus striatus, one of the most abundant reef fishes in French Polynesia, is the subject of a study being undertaken by the University of Hawaii (Hawaii Institute of Marine Biology) in conjunction with the Institut de Recherches Médicales Louis Malardé with reference to a number of aspects of the biology of this Acanthurid fish and with emphasis on its ciguaterix properties. A field laboratory was constructed and staffed at Atimaono, Tahiti, specifically for this research.

Ctenochaetus striatus, characterized by a single folding spine on each side of the caudal peduncle, faint longitudinal striations, and numerous elongate teeth loosely attached to the jaws, abounds in areas of suitable coral growth up to 35 meters, although it is most abundant in depths above 20 meters. The shallow water habitat is characterized by abundant *Acropora* coral growth with numerous sheltered surfaces for the species' quiescent night-time period. The fish is generally herbivorous, its fine dentition limiting the diet to fine algae and organic detritus. Feeding nearly ceases during the spawning season from November to March. The spawning process appears to consistently coincide with outgoing currents, understandably, as the egg and larval life of the species are pelagic.

Through SCUBA observations of tagged fish, it has been found that *Ctenochaetus striatus* undergoes a diurnal migration of 300-400 meters horizontally and 10-15 meters vertically to the reef edge. Some populations are involved in movement from a deep water resting place to a shallow reef feeding grounds, while other populations undergo the opposite migration. Parallel reef migration appears to be within 300 meters for this species of Acanthuridae.

Predation upon *Ctenochaetus striatus* is extensive, as it is preyed upon in all of its life stages - from egg to adult. While predators feeding upon the eggs and larval stages of *Ctenochaetus striatus* are normally non-toxic, those feeding on the adult fish are often toxic. The seasonal variation and the distribution of toxicity of *Ctenochaetus striatus* is being emphasized and will be discussed at the Tahiti Seminar on Ichthyosarcotoxism.

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SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16 - 22 August, 1968)

CIGUATERA IN QUEENSLAND

[Some notes on the Ciguatera syndrome as observed in Queensland over the last four years, particularly to record some of the features that do not seem to have been emphasised elsewhere.]

by

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"Ciguatera" appears to be as frequently encountered along the Great Barrier Reef as elsewhere in the Pacific. Generally its effects seem less severe, though this may be due to dietary preferences, e.g. the Acanthuroidea, although one of the commonest reef fish, is not generally eaten. Lutjanus nematofhorus and L. coatesi have a reputation for toxicity and are usually discarded.

More than half the cases are caused by Plectropomus maculatus, half the remainder by Epinephilus fuscoguttatus and tauvini with cases from Cheilinus undulatus, L. coatesi and nematophorus and Scomberomorus commeson. The average weight of the fish was fourteen pounds (6.4 Kilos).

Eighty cases of Ciguaetera caused by Scomberomorus commeson from Cairns, Townsville, Rockhampton and Gladstone have been recorded. No cases resembling "Scombroid" poisoning have been reported.

One case of Ciguatera due to Lates calcifer caught in an estuary south of Townsville was noted. This fish is most commonly found in tidal estuaries and inland freshwater streams, extending occasionally to inshore reef areas. It is not found on the Barrier Reef.

A most striking feature of Ciguatera here is its potentiation and reactivation by the ingestion of alcohol. Usually this causes skin itch, followed by paraesthesia of the hands and mouth as soon as five minutes after ingestion. It has occurred up to six weeks from the last fish meal.

Sensitisation or "memory effect" following Ciguatera is common. Usually for several months afterwards a small amount of similar fish non-toxic to a non-sensitised individual, will reproduce symptoms within a few hours. An extreme example followed severe poisoning from the liver of Epinephilus tauvini. This patient asserts that, seven years later, eating small amounts of Plectropomus maculatus or Epinephilus sp. still brought on recurrence of paraesthesiae and malaise, although Lethrinus chrysostomus from the same reef areas

causes no trouble. This fish, although one of the most common food fish is a rare source of Ciguatera. Recurrence of symptoms has occurred unexpectedly after eating such unlikely substances as anchovy paste, canned tuna and a local estuarine fish Otolithes argenteus.

Estimations of serum and red cell cholinesterase on cases in the Townsville area failed to show any tendency to a reduction, and trial of Neostigmin in affected persons was no better than placebo effect.

It would seem that while apparently a most laborious and exacting procedure, the isolation of toxic substances from the offending fish cannot be an end in itself unless the toxin can be proven to possess the capacity to reproduce the bizarre and exotic features of Ciguatera.

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16 - 22 August 1968)

ABSTRACT

CEREBELLAR SYNDROME IN CIGUATERA

Notes on two cases

by

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and

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At the Territorial Hospital in Papeete, the authors observed two cases of ciguatera presenting a new feature: to the usual clinical picture was added a total and severe bilateral cerebellar syndrome which developed slowly but favourably towards complete recovery without any after-effects.

The fish involved in each case belongs to the species Scarus microrrhinus Bleeker, its local name being "Uhu raepuu".

After describing the clinical symptoms, giving the results of the complementary tests that were carried out and outlining the treatment applied, the authors stress how difficult it is to diagnose a cerebellar syndrome which is not well-marked without a thorough neurological examination.

A parallel is then established with the symptoms observed in a series of cases of poisoning by Tridacna maxima on the island of Bora-Bora and it is emphasized that one of the toxic fishes was caught in the same "evil" area of this leeward island.

Finally, an attempt to elucidate the cause of the disease through a clinical approach is made in the light of present knowledge of the feeding habits of both Scarus microrrhinus and Tridacna maxima.

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

PHARMACOLOGICAL REACTION OF THE RAT TO CIGUATOXIN

by Martin D. Rayner*

The following ciguatoxin extracts, from flesh and livers of Johnston Island eels, have been tested by intravenous injection in rats:

(a) Flesh toxins

(i)	solvent extracted toxin, toxicity	600 γ/g
(ii)	column purified toxin	5 γ/g
(iii)	TLC purified fraction 1	0.5 γ/g
(iv)	TLC purified fraction 2	0.7 γ/g

(b) Liver toxins

(i)	column purified toxin	0.2 γ/g
(ii)	TLC purified toxin	0.03 γ/g

The effects noted can be classified as follows:

(a) Indirect or Reflex actions brought about as a result of the stimulation of certain peripheral receptors involved in cardio-vascular and respiratory control systems (notably the chemo and baroreceptors and the lung stretch receptors).

(b) Direct actions of the toxins, in particular

(i) CNS effects. Transitory excitation of respiration is followed by pronounced depression leading to total respiratory failure. Other systems are presumably also affected but have not yet been studied.

(ii) Neuromuscular effects. Increased tone and facilitation are followed by depressed excitability and eventually by complete neuromuscular block.

(iii) "Direct" cholinergic effects, e.g. Bradycardia and negative inotropic action;
Stretch and chemoreceptor stimulation;
Stimulation of intestinal smooth muscle;
Weak ganglionic stimulation.

(iv) "Indirect" cholinergic effects, e.g. Release of catacholamines from sympathetic nerve endings, resulting in peripheral vaso-constriction, tachycardia and positive inotropic action.

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These responses appear very similar to the effects of acetylcholine and nicotine; parallel *in vitro* studies have shown competitive interaction between ciguatoxins and nicotine at some sites. However not all of these actions are produced by all the listed toxin types (although different toxins prepared by the same procedure appear identical). It is noticeable that the least purified toxins affect the greatest number of sites. We conclude that the crude toxin contains more than one toxic molecule but that all toxin types have a basically similar "cholinergic" mechanism of action.

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

LIVER TOXINS FROM GYMNOTHORAX JAVANICUS

by Takeshi Yasumoto and Paul J. Scheuer*

For the past several years our source of ciguatoxin has been the flesh of moray eels, Gymnothorax javanicus, from Johnston Island. Because of our earlier unsatisfactory experience with the extraction of toxin from shark livers we had concentrated our efforts on the processing of eel flesh. A trial run with some eel livers unexpectedly showed that these livers can be extracted without difficulty and that they yield 2 ppm of toxin based on wet weight with an LD_{100} of about 0.05 mg/kg (IP injection in mice). Furthermore, we have been able to show that eels with a zero mongoose rating bear toxic livers of a toxicity comparable to those derived from eels with a mongoose rating of +3. Gross symptoms in mice injected with Gymnothorax liver toxin parallel those which are caused by injection of toxin derived from Gymnothorax flesh.

Chemically, intact liver toxin differs from flesh toxin by the absence of a glycerol fatty acid ester moiety. Until this point was established we carried out our degradative studies by hydrolytic procedures, which have been successful with flesh toxin. It now appears that hydrolytic methods are not suited to the nature of liver toxin and different approaches will be explored.

The chemical and physical properties of liver toxin essentially parallel those of the nitrogenous moiety of flesh toxin which is obtained by methanolysis followed by chemical reduction and acetylation. The low volatility of the toxin has so far hampered its investigation by mass spectral procedures.

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

A NEW FIELD AND SCREENING BIOASSAY FOR CIGUATERA

by

Emile Keene, Helen A. Randall and Albert H. Banner*

Field studies on ciguateric fish have been limited by the lack of a simple test for toxicity. To date, our knowledge of the chemistry of the toxin has not permitted us to develop any chemical test; in bioassay, screening tests with cats or mongooses are limited by the scarcity or absence of the animals on most islands while the mouse injection bioassay calls for long and elaborate chemical extraction of the toxin.

We have developed a new test, utilizing the stream crayfish of southern United States (Procambarus clarkii (Girard)) and an emulsified homogenate of the liver. The crayfish was chosen as it has twice been reported to be sensitive to toxic fish; the liver was chosen as it may contain up to 50 times as much toxin per unit weight as the flesh.

In the testing technique:

1. The liver is minced and then homogenized mechanically in a solution of 4.5% Tween 60 (Polyoxethelene sorbitan monosterate) in 0.9% NaCl, at the ratio of 1 g liver to 2 ml solution.
2. Heat homogenate to 60° C for 30 minutes; centrifuge.
3. Inject crayfish at 0.33% body weight on ventral side of second abdominal segment, lateral to midline.

If the crayfish is injected with a blank control solution, or with extract from nontoxic fish, there is no reaction. If the crayfish is injected with extract from a toxic fish, it reacts in the following ways:

1. Transient convulsions with abdominal flexture.
2. Loss of aggressive posture with the chela.
3. Awkward stance and twitching of appendages.
4. Loss of righting reflex.
5. Moribundity and death.

Of these reactions the one easiest to quantify is the loss of righting reflex. The measure of this is when the crayfish turns over or is turned to his back with a rod; if he fails to right himself after two minutes, the reflex is considered lost.

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Considerable variation was found in the crayfish; as it seemed to be unrelated to homogeneity of the injectant, to size, sex, presence of chela of the crayfish, or external physical conditions such as temperature; the variation was considered to be that inherent in a wild population. Statistical tests have shown that the average time to loss of righting reflex in a sample of five crayfish will differentiate between moderately and highly toxic crayfish. The dose-response curve is normal. Moderately good correlation is found with the results of the mongoose test, and initial evidence indicates a correlation with the amount of toxin recoverable from the flesh.

The test was developed using toxic moray eels, *Gymnothorax javanicus*, from Johnston Island. Ten other species of fish from other islands also showed correlations with the mongoose test. In *Ctenochaetus striatus* (Quoy and Gaimard), the "maito" from Tahiti, the reaction was so immediate and violent we suspect that it may have been caused by another toxin, the presence of which has been indicated by previous studies.

As the crayfish is not available in most archipelagoes, and as the stream shrimp, *Macrobrachium*, is found on most high islands, the results with the crayfish were compared to the reactions induced in *M. rosenbergi* De Man (A Malaysian species introduced to Hawaii). Here in eight tests using eels,"maito", and red snapper, there is a reaction qualitatively, and probably quantitatively, similar to that of the crayfish (the lack of enough *Macrobrachium* so far has prevented statistical correlation).

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

CIGUATOXIN

by Wataru Takahashi and Paul J. Scheuer*

Ciguatoxin as isolated from the flesh of Gymnothorax javanicus is a labile yellowish oil with an LD_{100} of 0.1 - 0.5 mg/kg (intraperitoneal injection in mice). It lacks distinctive ultraviolet absorption, but its infrared spectrum reveals the presence of hydroxyl and carbonyl moieties. Functionality tests fail to detect the presence of primary amino groups but indicate the presence of a quaternary amine moiety. Elemental analyses suggest an approximate empirical formula of $C_{35}H_{65}NO_8$ thus indicating a highly oxygenated molecule.

Mild acid or base hydrolysis liberates glycerol, fatty acids, water-soluble and organic soluble amine products. Gas-liquid chromatography reveals that the fatty acids are mixtures of which the major components are myristic, palmitic, palmitoleic, stearic, and oleic acids. High resolution mass spectral measurements of the water-soluble products indicate a large molecule with three nitrogen atoms. The organic soluble degradation product contains a quaternary amine function.

Oxidation followed by vigorous acid hydrolysis resulted in additional products. One of the fragments is non-nitrogenous and contains a linear hydro-carbon chain of about thirty carbon atoms. Another degradation product contains a quaternary amine moiety attached to a large alkyl group. A number of water-soluble amine fragments were also isolated. Chemical degradations and mass spectral measurements indicate that ciguatoxin is a large complex molecule in which the nature and exact number of nitrogen atoms are not yet known.

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

MARINE ALGAE AS A POSSIBLE SOURCE OF CIGUATERA TOXINS

by John E. Randall*

Several authors have suggested that benthic marine algae may be the source of the toxin which causes ciguatera. Knowledge of the food habits of fishes provides us with the strongest evidence of this. Some fishes which feed only on benthic algae may produce ciguatera when eaten. This does not constitute proof of an algal origin of the toxin, however, for herbivorous fishes often ingest detrital material of sundry origin and some fragments of animals incidentally with their plant food. Thus some other organisms such as a sponge or a fungus might be the causal agent. Or it might be an organism such as a bacterium growing in association with an alga. Another reason to suspect algae is that toxic substances have been isolated from them (although none yet resemble ciguatoxin).

Algae were collected from reefs where poisonous fishes are found in the Society Islands, especially those which grow early on new surfaces, for testing of toxicity, but none displayed any significant quantity of toxin. The first alga tested was the chrysophyte Chrysonephos lewisii; it was clearly the dominant organism on surfaces of concrete, coral rock, and durotex placed on three toxic reefs in Tahiti and one in Moorea during the southern winter after a period of two to three weeks. The green alga Enteromorpha sp. and the brown Ectocarpus sp. succeeded Chrysonephos on the new surfaces after about a month in the sea. Still later these algae were largely replaced by many species such as the bluegreens Calothrix crustacea and Lyngbya majuscula, the brown Sphacetaria sp., the green Cladophora sp., and the reds Polysiphonia sp., Centroceras clavatum, and Laurencia sp. These algae were tested en masse. The bluegreens Hormothamnion enteromorphoides, Lyngbya aestuarii, and Calothrix sp. and assorted diatoms were the principal algae growing on a concrete surface placed on a sand bottom in the lagoon near a toxic reef in Tahiti; this surface was subject to some silting by sand. A new surface placed in the sea on December 23 for twenty days yielded primarily Cladophora, and one in 15 days beginning February 29 mainly Enteromorpha (with some Eryptotrichia, Sphacelaria, and Cladophora). The bluegreen Phormidium sp., which was found growing naturally at the edge of Teavaraa Pass, Tahiti, was also tested. The two mice injected with extracts of this alga died in 34 minutes at the dosage of 2000 γ/g. Deaths from other algae required at least 3000 γ/g. A test with seawater alone produced death in two mice in 85 minutes at 3500 γ/g.

The following algae were collected at toxic sectors at Ishigaki in the Ryukyu Islands: the green Chlorodesmis sp., the diatom Synedra sp., and the bluegreens Phormidium sp. and Lyngbya sp. None were toxic beyond the level of 300 γ/g.

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Although these results do not suggest that any of the algae tested are the source of ciguatera toxins, it should be remembered that the toxic level in the responsible organism may be very low and thus difficult to demonstrate by direct feeding or injection of extracts into mice. Also it is possible that a precursor of the toxic substance occurs in an alga which exhibits no toxic properties until it is metabolized by animals.

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SPC/ICHT/WP.21
16th August, 1968

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Papeete, French Polynesia, 16th - 22nd August, 1968)

ABSTRACT

PATTERNS OF DEVELOPMENT OF CIGUATERA FISH POISONING IN THE PACIFIC

by Philip Helfrich*

The classification of fishes that are toxic to eat have been grouped in various ways and, for the purposes of this discussion, a modification of the classification of Halstead (1967) is followed. It should be noted that ciguatoxic fish or those which cause the disease "ciguatera" when ingested by humans may be the result of more than a single toxin; at least four toxins have been implicated in ciguatera outbreaks described by Banner (1967). In this discussion of the patterns of development of ciguatera no attempt will be made to distinguish the various toxins that might be involved in outbreaks of the disease.

Fluctuations in the geographical distribution and intensity of ciguatera fish poisoning have been experienced in several areas in the Pacific, as reported by Jones (1956), Cooper (1964), Bartsch, et al. (1959), Helfrich and Banner (1964), and Helfrich, et al. (1968). An examination of the available information suggests a sequence of events that typify an outbreak or marked increase in cases of ciguatera in a given area. The phenomena triggering such outbreaks are largely unknown, however the hypothesis developed by Randall (1958) suggests mechanisms whereby the sequence of events in fluctuations in ciguatera might be explained. Randall, expanding on the work of earlier researchers, hypothesized that the toxin was produced by a benthic organism, probably an alga or organisms living on an alga, which underwent expotential type growth under certain conditions. He suggested that at least one of these conditions was the exposure of new surfaces on the submarine substrata.

Based upon Randall's hypothesis and upon the information available on outbreaks, although such information is quite inadequate, a scheme of trophodynamics of the toxin in an ecosystem is proposed.

Examination of data on fluctuations of ciguatera from islands and archipelagoes of the Indo-Pacific appear to fall into three categories: I - Pattern of intoxications observed in areas with no previous history of ciguatera; II - Pattern of intoxications in areas with a continuous history of ciguatera at a low level, with periodic marked increases in ciguatera; III - Pattern of intoxications at a sustained low level with well defined geographical limits and species involved.

The dynamics of these three patterns is proposed.

The patterns of ciguatera development hypothesized are based upon incomplete evidence from personal observations and various scattered sources. The evidence for their existence is somewhat tenuous and conjectural, however it is presented as a possible framework to which further evidence can be added to aid us in understanding the dynamics of ciguatoxin in marine communities. It is hoped that at least it will stimulate fruitful discussions that will open up previously unexplored areas of research.

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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
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ABSTRACT

CIGUATERA-TYPE FOOD POISONING ON THE ISLAND OF REUNION (1966-67) AND J. MORICE's THEORY REGARDING THE ORIGIN OF THE TOXICITY OF SOME TROPICAL FISH SPECIES

by

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1. On the Indian Ocean island of Reunion, the consumption of tropical fish caught either locally (by small fishermen) or on the banks of Saya de Malha, some 700 nautical miles to the north, caused several cases of ciguatera-type poisoning. The species involved are named; they are mainly Serranidae, Lutjanidae and Caranqidae. The actual incidence of fish poisoning has not yet been estimated.

2. A possible explanation of the toxicity of some tropical fish species is given. Although no systematic testing of this hypothesis has yet been undertaken, the author deems it worthwhile to summarize the observations made from 1961 to 1964 by Morice on St Bartholomew Island (French West Indies). These suggest that the toxicity of some of the fish might be connected with the presence of skull lesions, severe in some cases. Such lesions may be caused by a micro-organism carried by isopodous crustacea which are parasites of the branchial cavity and nostrils and are frequently encountered in many species. We believe we have found such lesions in fishes in the Indian Ocean and on the coast of Tahiti.

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SEMINAR ON ICHTHYOSARCOTOXISM
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NOTE ON THE USE OF OXIMES IN THE TREATMENT OF CIGUATERA

by Raymond Bagnis
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The use of oximes in the treatment of ciguatera was envisaged in 1965 following pharmacological investigations which had been carried out at the Hawaii Marine Institute and which had revealed, in the course of laboratory experiments on rats, that there was an analogy between the effect of "ciguatoxins" and that of organophosphorous products contained in certain insecticides.

The experimental product used was Protopam(R) chloride distributed by "Campbell Pharmaceutical Inc., New York", the chemical formula of which is as follows: 2-formyl-1 methyl pyridinium chloride oxime. This patent drug is regarded as having no serious secondary effect.

When administered in conjunction with atropine, "Protopam" keeps alive rats who had been given twice the normal lethal dose of impure ciguatoxin.

It was used in the treatment of humans for the first time in September 1964 in Honolulu, in the case of one of the victims of an extremely serious intoxication which had already caused the death of two people. Whilst the results could not be described as conclusive, the way was open to further experiments. Unfortunately in view of the absence of other acute forms of ciguatera, American research-workers were unable to use this product more extensively in Hawaii itself.

In the light of these data and for a period of approximately six months during 1966 ciguatera was being treated experimentally in Tahiti by means of "Protopam" - a procedure carried out side by side with the symptomatic treatment which was still the only one applied in most cases. Analysed below are our results bearing on 48 patients:

Means of administration: intravenous or intrasmuscular.

Method of application: on its own or in conjunction with atropine.

Doses: Adults : 1 gramme injection which may be repeated.

Children: 25 mg injection per kg of body weight.

Number of patients treated: 48

Form of procedure used in experiments on adults:

Method 1 : Very serious cases of immediate urgency, accompanied by a state of shock.

- 1 gramme of Protopam in an intravenous perfusion of 250 cc of salt serum to be given in the course of 30 minutes.
- ¼ milligrammes of atropine to be given subcutaneously and repeated within the following half-hour.
- These doses may be repeated once after two hours if the state of shock remains unchanged.

Method 2 : Serious cases unaccompanied by a state of shock.

- 1 gramme of Protopam in 20 cc of distilled water given by means of a very slow intravenous injection (at least 2 minutes).

- For the atropine, some technique and dose as in Method 1.

Method 3 : Average cases.

- 1 gramme of Protopam in 10 cc of distilled water given by means of a slow intramuscular injection (one minute) either on its own or in conjunction with ¼ of a mg of atropine given subcutaneously.

RESULTS

Method 1 : - 5 cases treated in Hospital

3 caused by Lethrinus miniatus
2 caused by Lutjanus gibbus

- Spectacular effect on the digestive symptoms, the cardio-vascular symptoms and the chills.

- Less rapid effect on the neurological symptoms

Method 2 : - 5 cases treated in Hospital

2 caused by Lethrinus miniatus
3 caused by Ctenochaetus striatus

- Remarkable effect on the entire clinical picture in the 4 cases where the patients were seen less than 10 hours after the appearance of the first symptoms.

- Effect far less clear-cut in the 5th case seen at a much later stage (48 hours after).

Method 3 : - 38 cases treated : 37 out-patients - 1 in Hospital

- In 21 cases, given in conjunction with ¼ mg of atropine administered subcutanenously.

- Results very variable and difficult to analyse, in view of the frequently prolonged latency period (48 hours and over) between the appearance of the first symptoms and the time of consultation, and the scarcity of symptoms which could be identified objectively during a clinical examination.

However, here again, success depends on the speed with which treatment is applied rather than on the causal species.

The digestive symptoms disappear; the neurological symptoms subside, but do not vanish immediately; the pruritus remains.

Protopam is more effective when used in conjunction with atropine.

CONCLUSIONS

Being a drug with a particularly favourable effect on the symptoms provoked by the excessive vagal stimulation characteristic of the muscarine-type phase of ciguatera, Protopam becomes more potent when used in conjunction with atropine.

Its action is the more effective if administered early and, in serious cases, immediately.

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Original Text: French

ANNEX IV a

Message from the Secretary-General of the South Pacific Commission

It is with pleasure that I send a message, through Dr A. Guinea, the Commission's Medical Officer, to welcome the participants to this Seminar on Ichthyosarcotoxism. Dr Guy Loison, the Commission's Programme Director for Health, joins me in expressing warm appreciation to Tahiti for extending its traditional hospitality so that the Seminar could be held here. It is appropriate that this is so as there has been a considerable amount of research in this area. The subject of fish poisoning is an important one and it is almost certain that some of you here have had personal experience of it. I am sure, therefore, that your discussions will be followed closely and with great interest.

The fact that toxic effects can result from eating fish has been known for a very long time by the inhabitants of the islands in the Pacific. The people who live from the sea have acquired considerable knowledge on poisonous fish and have evolved certain procedures to judge whether or not a certain fish was poisonous. But this has not, any more than it does not today, prevent widespread illness and even death as the result of fish poisoning.

It is only recently that the scientist has caught up with the indigene in his observations and that there has been any attempt to investigate the phenomenon of toxicity as the result of eating fish. Interest, however, has been increasing in recent years as it became obvious that this problem involved much more than individual illness or death. Some populations in the Pacific area are growing at an estimated rate of up to 4.6% per year. With limited land area and limited land utilisation, the protein assets available in surrounding seas become increasingly important. Changing cultures, changing economics bring changed pattern on nutrition but the desire and the need for fish protein remains. If this need is to be met, it is of the utmost importance that the nature of fish poisoning be understood in an effort to find some solution. The Commission has been interested in and aware of the problem since 1950. From that time it has aided in research and the accumulation and distribution of as much relevant information as possible on the subject by the provision of technical services and financial assistance. In the same period, many individual scientists from numerous disciplines have maintained research in an attempt to find rational answers to a problem that seems essentially irrational. With so many variables, this multidisciplinary approach is essential; the doctor does not always know a great deal about marine plants, or the involved chemistry of toxins isolated. If success can be attained it is necessary that the information obtained by medical officers, marine biologists, chemists, ecologists, oceanographers and other specialists be correlated and discussed. This, of course, is the object of the Seminar and the Commission is proud that it has been of assistance in organizing this, the first such Seminar in the South Pacific.

It is hoped that the meeting will produce some recommendations which will be of value to the great many people living in the area and I hope that the discussions will also assist each member of the Seminar in his own endeavours.

The Commission will be grateful if the participants make some of their recommendations available for suitable action and it will be happy to receive requests from territories for the services of consultants or for study tours for local officers to attend places of research. In addition, the Commission will be glad to publish information on fish poisoning and to arrange future seminars.

Finally, I would like to thank the Government of French Polynesia, His Excellency the Governor, members of the Conseil de Gouvernement and the people of Tahiti for their hospitality. I would also like to acknowledge the assistance of the Director of the Institut de Récherches Médicales de Polynésie Française, of the Director of Health and of Dr Bagnis who has worked so hard as liaison officer for the Commission in the arrangements for this Seminar.

Original Text: English

ANNEX IV b

Opening Address by the Secretary-General of French Polynesia

Sir, (to the South Pacific Commission Representative)

Ladies and Gentlemen,

Governor Sicurani has been detained with the Minister for Scientific, Space and Atomic Research who arrived in Tahiti this morning, and he has accordingly asked me to represent him and to tell you that he is extremely sorry to be unable to preside in person over this Opening Session of the Seminar on Ichthyosarcotoxism.

In his name I bid you welcome to French Polynesia.

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A little over a year ago, in May 1967, a Conference was held in Tarawa under the auspices of the South Pacific Commission on the special aspect of food and health problems of coral atoll populations.

In accordance with the discussions a recommendation was adopted emphasizing the need to develop and co-ordinate research into the intoxications caused by the ingestion of certain marine organisms.

This meant stressing once again a very old peculiarity of certain fish species, confirmed by many a ship's crew, often at the members' own cost, and recorded in their log-books by all the great navigators who followed Cook, Wallis, Bougainville and La Pérouse.

I should like in particular to quote Moerenhout who was travelling to the Great Ocean Islands between 1829 and 1834, and who reports that, when calling in Hood Island - probably the island known to-day under the name of South Marutea, in the Tuamotu Group - he and his schooner's Captain and crew became the victims of poisoning after eating some fish, albeit perfectly fresh fish. "When I tried to get up", he writes, "I felt strangely unwell. A headache and pains in every part of my body scarcely allowed me to get out of bed."

I leave it to you to decide, Gentlemen, whether this excellent observer of Polynesian manners and customs has managed to give an accurate description of the symptoms of an illness which, having carefully considered all kinds of hypotheses, he attributes to the fact that "Certain fish which we thought wholesome, were poison".

What cannot be disputed is that this illness did exist and that the islanders knew and feared it, since Moerenhout further records, in connection with Waterland Island - doubtless the present Ahe Island - that "the Indians do not want to live there and even claim that, during various seasons of the year, all the fish in that area represent a poison pervasive enough to cause death within a few days."

Since the days of Moerenhout, we have scarcely progressed in our knowledge of this mysterious sickness, and the fear of poisoning to-day deprives the peoples of the Pacific Islands, the West Indies and some of the Far Eastern and Indian Ocean Island Groups of food resources which could be drawn in abundance from the surrounding Ocean.

On account of its economic and social repercussions, the problem of fish toxicity has in recent years been exercising the minds of an increasing number of research scientists.

The picturesque records of ancient navigators have given way to scientific observations. Yet the task is a difficult one, for the phenomenon under investigation - now known under the very learned name of Ichthyosarcotoxism - appears to vary in time and space, according to the persons who have partaken of the meal in question and the physiological reactions of the transmitting organisms.

French Polynesia, whose inhabitants live by tradition on the products of the sea, has been actively contributing to this research since 1961.

In 1963 an agreement of mutual co-operation was drawn up between our Medical Research Institute and the Marine Institute of the University of Hawaii, under which toxic fish or fish presumed to be toxic were collected by the Institute and despatched to Honolulu for toxicological analyses.

Then in 1966, on the instigation of the late Dr Malardé, a Medical Oceanography Section was set up in the Research Institute.

To mark its profound interest in Ichthyosarcotoxism, and mindful of the recommendation put forward by the Tarawa Conference, our Territory - with the South Pacific Commission's technical and financial assistance, has organized this Seminar thanks to which the scientists and experts of world renown who were kind enough to accept our invitation and whom I am happy to welcome here will be able to discuss their work, compare results and, we hope, extend our knowledge of this endemic disease.

The studies you will undertake will doubtless not be strictly limited to the scientific aspect. You will examine the medical, social and economic consequences of Ichthyosarcotoxism. Your research will fall into the wider framework of the overall investigation of the lagoon environment and the rational utilization of marine resources. Thus a very vast subject is being placed before you.

Your discussions will be carefully followed by a youthful audience whose members will be interested, at times passionately interested, for our islands' calling for the sea leads many of our students to turn towards subjects connected with the marine environment, such as marine biology, physical oceanography and allied sciences.

In a few years' time these young people, the audience of to-day will take part in your work and form the nucleus of the research scientists who will open up new vistas for this territory.

The beauty of our landscape, the calm of our lagoons, Tahitian hospitality, these are our tourist arguments. They will be appreciated, I am certain, and turned to the best possible advantage by discriminating tourists such as yourselves, for the value of any scientific discussion can only be enhanced if it is set in a pleasing framework. This is why we feel that Tahiti, the cross-roads of air-routes in the centre of the Pacific, can nurture the ambition of becoming a focal point of scientific and cultural activity open to the exchange of ideas, and the seat of applied research organizations offering to foreign and to its own scientists all the installations required to provide the optimum living and working conditions.

Thus to the name of Tahiti, in addition to the renown for its traditional charms, will be attached all the prestige of the Science of Humanity and of Disinterestedness.

Gentlemen, I wish you a pleasant stay amongst us, and I declare open the Seminar on Ichthyosarcotoxism.

Original Text: French