SOUTH PACIFIC COMMISSION

AD HOC ADVISORY GROUP MEETING ON FISH POISONING
(Papeete, Tahiti, French Polynesia, 15 - 18 February, 1977)

I. INTRODUCTION

This meeting, held at the library of the Institut de Recherches Médicales "Louis Malardé", Papeete, Tahiti, had as its goal the sharing of recent results of studies on ciguatera which have been supported, in part, by the South Pacific Commission. The institutions where this work is being conducted include the Tohoku University, Sendai and Kagoshima University, Kagoshima, Japan, the University of Hawaii, Honolulu and the Institut de Recherches Médicales "Louis Malardé" (IRMLM), Papeete, Tahiti.

2. PARTICIPANTS

Dr. J. Laigret, Director, IRMLM, Papeete.

Dr. A. Bourre, Medical Officer, South Pacific Commission, Noumea.

Dr. A.H. Banner, Principal Investigator, Studies on Ciguatera, University of Hawaii, Honolulu.

Dr. L.R. Berger, Professor of Microbiology, University of Hawaii, Honolulu.

Dr. Y. Hokama, Professor of Pathology, University of Hawaii, Honolulu.

Dr. T. Yosumoto, Professor of Food Hygiene, Tohoku University, Sendai, Japan.

Dr. A. Inoue, Associate Professor of Marine Botany and Environmental Science, Kagoshima University, Kagoshima, Japan.

Dr. R. Bagnis, Head, Unit of Medical Oceanography, IRMLM, Papeete.

Dr. F. Parc, Pathologist, IRMLM, Papeete.

Mme E. Chungue, Biochemist, IRMLM, Papeete.

Mme S. Chanteau, Biochemist, IRMLM, Papeete.

M. J.H. Drollet, Biologist, IRMLM, Papeete.

3. AGENDA

STUDIES ON DIPLOPSALIS

Historical perspective.

Comparative toxicity of food chains.

Evidence for Diplopsalis as a likely cause of Ciguatera.

Biochemical properties of various toxins from Diplopsalis recovered from coral surfaces.

The culture of Diplopsalis.

Establishment of a survey method for Diplopsalis.

Distribution of Diplopsalis around Tahiti.

OTHER MICROBIOLOGICAL STUDIES ON CIGUATERA

BIOCHEMICAL AND PHARMACOLOGICAL STUDIES

Toxins of Parrot Fish.

Pharmacological studies of toxins involved in Ciguatera.

Research on possible antidotes for the ciguateric intoxication.

IMMUNOLOGICAL STUDIES

Radioimmunoassay for the detection of ciguatoxin.

Preliminary immunofluorescence data on Diplopsalis

Hypersensitivity in Ciguatera in cats and epidemiological incidence in various territories of Pacific area.

4. RESULTS

4.1 RECENT EPIDEMIOLOGICAL DATA FROM THE SOUTH PACIFIC AREA

4.1.1. Health Incidence

An attempt has been made to know the present state of ciguatera in the South Pacific area from the official number of fish poisoning cases reported to the South Pacific Commission. From December 1975 to November 1976, inclusive, approximately 2,000 cases have been officially recorded. This number shows a 10% increase over those of 1975, but after other studies we have made in Fiji, Niue, Tonga, Samoa, New Caledonia, New Hebrides and Loyalty Islands, we can certify that this number is minimal.

Nevertheless thanks to these data, we can get a general view of the disease in the various regions. It thus appears that the most affected territories are Tuvalu, French Polynesia, New Caledonia, Trust Territory of the Pacific Islands and Gilbert Islands.

4.1.2. Clinical Aspects

The general scheme described in French Polynesia can be easily extended to the other areas of the South Pacific. The ciguateric syndrome shows two major clinical pictures which seem to proceed from different physiopathological mechanisms. In the first, the food poisoning shows more or less severe digestive, nervous and cardiovascular symptoms. These are undoubtedly due to a pharmacological effect of ciguatoxin, when this product reaches a high concentration in the fish. In the second clinical form, the neurological syndrome is often isolated (including primarily dis-esthesia, pains and itching), and sometimes associated to diarrhea. It does appear to be related to some antigenic properties of ciguatoxin and involves an hypersensitization to fish. It does affect primarily individuals who continue to eat fish following intoxication with true fish poisoning. Most of the time, this syndrome occurs in remote places where islanders have not the opportunity or the goodwill to vary their diet. (Gambier, Marquesas and some atolls of Tuamotu archipelago). This allergic-like condition may become chronic and constitutes a true public health problem which is initiated by consumption of normally edible sea products. In Tahiti the number of cases apparently correlated with a hypersensitization has increased in the last few years. It accounted for 30% in 1975. An inquiry on patients treated at IRMLM has revealed that only 32% were poisoned for the first time. The others came for treatment of recurrences. In these patients there is often an overlapping between the pharmacological (direct or cumulative) and immuno-logical effects. On the other hand, in the hospital, during a severe state of shock, it is sometimes very hard to distinguish in the severe clinical picture, the part of each pathogenic pattern.

The hypothesis of an immunological participation associated with the toxicological effect is also supported by various incidences of asthma, urticarian reaction, eczema sprouting, vitilago, started by a typical fish poisoning.

4.1.3. Etiological "in situ" Patterns of Ciguatera

One can distinguish in the Pacific area two kinds of etiologic patterns for ciguatera. The first one is of an outbreak type. It implicates the complete benthic food chain, involving microphagous and detritivorous fish (surgeonfish, parrotfish) and their predators (groupers, snappers, emperors, moray eels, jacks barracudas) primarily. It is observed only in the areas suddenly affected by strong, limited, repeated disturbances (direct or second-ary human agressions). This picture, noted in the past decade in French Polynesia, is not too common. It is even rarer in many other territories. The second etiologic type implicates only the ichtyophagous fish, leaving the first trophic levels of the food chain entirely edible. It is commonly encountered in all the areas only when natural mechnical diffuse and episodic disturbances occur. It produces a low toxicity in the reef ecosystem.

This has been supported by tests of a few specimens of high trophic level fish from New Caledonia and Fiji which do carry little or no ciguatoxin.

4.1.4. Studies on Diplopsalis

A. Discovery of the etiological agent of ciguatera

An investigation on food chains of ciguatera revealed the presence of strong toxicity in a mixture of algae and detritus scraped off the surface of dead corals collected on the Gambier Islands. A large number of a dinoflagellate tentatively identified as Diplopsalis sp. nov. was present in this toxic sample. This organism is detected only in toxic samples but not in samples of low toxicity. This suggested a correlation between the organism and toxicity. Mechanical fractionation of the toxic sample by sedimentation and sieving enabled us to obtain a fairly uniform sample of this organism. Subsequent biochemical experiments based on various chromatographic methods showed the presence of two toxins which are very close to, or identical with, ciguatoxin and maitotoxin. Pharmacological and immunological tests done by Professors Rayner (Pharmacology) and Hokama at the University of Hawaii also offered evidence that the organism produces a toxin which is indistinguishable from ciguatoxin. All these data indicate that Diplopsalis sp. nov. is the cause of ciguatera.

B. Ecological survey

A rapid and practical method for surveying Diplopsalis sp. nov., the probable causative organism of ciguatera, was developed. The test was done by shaking 10 leaves of a brown alga, Turbinaria ornata, in a test tube containing 10 ml of sea water. After centrifugation of the liquid content the precipitate was re-suspended in 0.5 ml of sea water and two drops of the suspension were transferred on a slide glass. The number of Diplopsalis cells was counted under a microscope. If T. ornata was not available, one gram of another species of alga was used instead.

By using this method, the distribution of Diplopsalis was surveyed around Tahiti and Gambier Islands. A wide distribution of this organism in the surveyed areas was confirmed; also marked regional variation was observed. Periodical observation of numbers of this organism on a small reef indicated a remarkable rise and fall during the observation period. It is expected that this survey method will be applicable to other areas to assess the ciguateric endemicity.

C. Culture

Unialgal culture of Diplopsalis sp. nov. was achieved in enriched sea water medium by a micropipette procedure. The cell division was calculated to occur approximately once every three days. Essential vitamins for its growth were presumed to be vitamin B_{12}, biotin and thiamin.

4.1.5. Other Microbiological studies

Only one bacterial culture, "Bacillus 18", of the hundreds brought back in 1975 from French Polynesia and subsequently tested, reacted with anticiguatoxin sera. Anti-"Bacillus" rabbit sera reacted strongly with toxic reef fish, weakly with edible reef fish flesh from Hawaii and insignificantly or nil with pelagic fish. The conclusions were confirmed by

Professor M. Rayner using the above two sera and with sera modified by absorption with the non-homologous antigens, as blocking agents for the effect of ciguatoxin in a specific membrane test.

The establishment of whether "Bacillus 18" contains an antigenic determinant identical to the one in ciguatoxin is our current priority goal. An assay required to test fractions of "Bacillus 18" for anticiguatoxic reaction has been set up.

The identification of "Bacillus 18" as a member of a known but unnamed phenotypic group of marine bacilli is near completion.

4.2 BIOCHEMICAL ASPECTS

4.2.1 Studies on Scaritoxin

The occurrence of a new toxin tentatively named scaritoxin (SG_1) besides a ciguatoxin-like toxin (SG_2) in a parrotfish, Scarus gibbus, led us to investigate the distribution of these toxins in other species of parrotfishes and in other ciguateric fishes belonging to various genera. Abstracts of the results follow :

- Scaritoxin is present in all the parrotfishes studied : Scarus harid, Scarus niger, Bolbometopon bicolor, Scarus mus, Scarus ghobban and Scarus frenatus in significant amounts in addition to ciguatoxin-like compound (SG_2).

- Scaritoxin is absent in the following species : Epinephelus microdon, Plectropomus leopardus (both ichtyophagous carnivorous), Lutjanus bohar, Monotaxis grandoculis (both eclectic carnivorous) and Naso unicornis (herbivorous). Only ciguatoxin-like toxin was found in these fishes.

Furthermore conversion of ciguatoxin into scaritoxin is possible. The conversion does not take place in the liver but may occur in the muscle.

4.2.2 Toxicity of Biodetritic Layer Covering Dead Corals

To study the distribution of ciguatoxin-like and maitotoxin-like toxins on dead corals from Gambier Islands dead corals were scratched and fractionated by particle size by sieves and centrifugation. The highest number of Diplopsalis counted were in the fractions between 500 μ and 36 μ. The fractions obtained were extracted and two kinds of toxins were found : the maitotoxin-like (MTX) and the ciguatoxin-like (CTX).

In total toxicity, the MTX represents 71.9% while the CTX represents only 28.1%.

4.2.3 Comparison of MTX Diplopsalis - MTX Ctenochaetus striatus and CTX Diplopsalis - CTX Gymnothorax javanicus

The comparison between the two toxins of Diplopsalis and the toxins MTX of C. striatus (maito) and CTX of G. javanicus (moray eel) shows great similarities which have to be investigated in the future.

4.2.4 Relation Between Toxicity of C. striatus and the Population of Diplopsalis on the Reefs of Tahiti and the Gambier Islands

In six stations around Tahiti and one station on the Gambier Islands we found good correlation between the toxicity of C. striatus and the number of Diplopsalis. The highest toxicity in the fish corresponds to the highest number of Diplopsalis.

4.3 PHARMACOLOGICAL STUDIES

4.3.1 Muscle Membrane Actions of Ciguateric Toxins

Both liver and flesh ciguatoxins from Gymnothorax javanicus produce a characteriestic series of effects at muscle membranes. Initial addition of toxin to the bathing medium produces rapid depolarization of muscle membranes. Membrane potential can be restored to normal resting levels by an increase in Ca^{2+} concentration of the bathing medium.

This recovery is due to an antagonism of the toxin effect (rather than displacement of toxin from the membranes) since depolarization returns when the muscle is returned to normal saline solution. That these actions are mediated by effects on sodium permeability is indicated by the recoveryof resting potential following the addition of tetrodotoxin.

This same pattern of effects is also produced by the non-water soluble toxin from Diplopsalis, providing substantial evidence that this toxin should be regarded as pharmacologically identical to ciguatoxin isolated from moray eels. By contrast the water soluble toxin from Diplopsalis and both water and solvent soluble toxins from the parrotfish did not show this marked sensitivity to either tetrodotoxin or to high calcium concentration. We conclude that the Dipslopsalis "maitotoxin" and the Scarus gibbus toxins are pharmacologically distinct from the principal moray eel toxins.

4.3.2 Effects of Various Drugs on Death Times of Poisoned Mice

To look for possible antidote effects against CTX we have tested different drugs injected intraperitoneally into mice. The injection took place one hour before, concomitantly, or one hour after the CTX injections. Various dosages of drugs and toxins were tested. Most of the time we have used methanolic residues of S. gibbus muscle as CTX. The experiment drugs have been atropine sulphate(anticholinergic type), calcium gluconate and glutamate (cell membrane stabilizing type), pralidoxin (cholinesterase reactivator), dexchlorpheniramine (antihistaminic type), betamethasone (corticoid type), diethazine chlorhydrate (parasympatholytic type), tetrodotoxin (specific experimental CTX antagonist).

From the results that we have obtained, the calcium glutamate at a dosage of 0.02 g per 20 g mouse has slightly delayed the death time. But the most significant effect has been involved by tetrodotoxin. This drug, at a 1/2 ml dosage, has not only delayed the death time, but has also given a 30% protective effect against lethality when the totality of controls die. Atropin sulphate has shown to decrease the diarrhea exhibited by poisoned mice.

All the other drugs, including cholinesterase reactivator, antihistaminic and corticoids, were inactive.

4.4 IMMUNOLOGY

4.4.1 Radioimmunoassay

A practical, sensitive, simple, and relatively specific radioimmunoassay test for the assay of ciguatoxin directly from natural sources has been developed. The radioimmunoassay test has been utilized to distinguish the toxic fishes identified in human ciguatera poisoning outbreaks from the non-toxic fishes. The radioimmunoassay test showed a fair correlation with the mongoose assay in the analysis of eel tissues ($r = 0.54$). It is suggested that further studies should aid in the development of a practical, sensitive, simple and specific immunological test for routine examination of ciguatoxin in fishes and for the analysis of ciguatoxin structure.

4.4.2 Experimental Hypersensitivity in Ciguatera

Several cats were hypersensitized by successive oral fish poisoning and tested by localized passive cutaneous anaphylaxis. Hypersensitivity reaction was demonstrated only following several intoxications. A large amount of homologous toxin was required.

5. INTERNATIONAL EXCHANGES

5.1 SPONSORED BY THE SOUTH PACIFIC COMMISSION

Ad Hoc Advisory Group meeting on Fish Poisoning (Papeete, 5 - 9 January, 1976).

Ad Hoc Advisory Group meeting on Fish Poisoning (Papeete, 15 - 18 February, 1977).

5.2 SPONSORED BY WORLD HEALTH ORGANIZATION

Dr. T. Yasumoto (University of Tohoku, Sendai, Japan) acted as World Health Organization consultant in biochemistry from 30 December, 1975 to 20 February, 1976 at the IRMLM, Papeete, Tahiti. He has just completed another co-operative study with the IRMLM from 30 November, 1976 to 11 February, 1977.

6. CONCLUSIONS AND PERSPECTIVES

During the past three years the support of the South Pacific Commission has catalized research on the widespread disease, ciguatera, by three independent but co-operating groups : those in Hawaii headed by Dr. Banner, those in Tahiti headed by Dr. Raymond Bagnis and those in Japan headed by Dr. Takeshi Yasumoto. Because of this support the three groups have been able to obtain additional funds from their various national and international agencies.

Their combined research has produced results that will be far-reaching in the health and economics of the South Pacific Islands. Research at the University of Hawaii has given investigators two immunological tests to find and follow ciguatoxin on the coral reefs and in laboratory study. One of these tests, a radioimmunological assay, will also enable laboratories to test with small expense all potentially toxic fishes in commercial fisheries so that the island people may eat with safety the fishes of their waters. Probably more important for the future is the isolation and cultivation of the toxigenic species on the coral reefs. This one-celled plant known as Diplopsalis releases into the food chain of the reef those quantities of ciguatoxin and other toxins that finally occur in both herbivorous and carnivorous fishes. It was discovered in the toxic reef areas of the Gambier Islands by Dr. Bagnis' and Dr. Yasumoto's groups, working together. The understanding of the ecological requirements of this toxigenic species can be expected to lead to the prediction of outbreaks, the confirmation of the limits of toxic areas, and possibly even the control of outbreaks.

While these advances have been great, further work must be done in the present year and possibly three or more years to come. The active research groups will continue to seek the bulk of their research support from other sources, but their studies will be facilitated again by the aid of the South Pacific Commission in certain co-operative aspects.

7. RECOMMENDATIONS TO THE PROJECT PARTICIPANTS

7.1 Establishment of a Testing Programme for Potentially Toxic Commercial Fish by the Radioimmunological Assay

Dr. Hokama of the University of Hawaii will launch a programme for the testing of the amberjack (Seriola dumerili), a fish now banned from the market, in co-operation with the Hawaii State Board of Health. Concurrently, he will apply the test to samples sent from Tahiti. After the programme is established, he will train in the procedure a technician sent from Tahiti.

7.2 Initial Exploration of Methods of Treatment of Patients with Ciguatera

Dr. Parc, of the Institut de Recherches Médicales "Louis Malardé" in Papeete will explore recently discovered leads in immunology for the treatment of the disease.

7.3 Development of Techniques for Pure Culture of Diplopsalis

Drs. Yasumoto and Inoue will complete development of techniques for pure culture of the dinoflagellate when they return to Japan. They anticipate this will be done within the year. When they are successful they will culture the form for points 7.4 and 7.5 below.

7.4 Study of Ecological Requirements of Diplopsalis

Through different culture media and laboratory conditions, the Japanese will study the effects of light, heat, salinity, nutrients, trace organics and inorganics etc. upon both the growth and toxin production of the cultures. Concurrently, the Bagnis group in Tahiti will monitor sea conditions and growth of the plant on local reefs. Dr. Hokama in Hawaii will do these investigations by testing samples with his radioimmunoassay. These two sets of observations, when combined, should lead to the ability of scientists to predict, delimit, and possibly control outbreaks, as mentioned in the introduction.

7.5 The Mass Culture of Diplopsalis

Fundamental studies on the toxin heretofore have been limited by the extremely small amounts of pure toxin produced at great effort and expense. Through modern mass culture techniques, the plant and the toxin it produces can be harvested at little effort. The mass produced toxin, when purified, will be used for :

(a) Immunological tests

The availability of highly purified toxin will permit very specific immunological assay needed for research.

(b) Structural chemistry of the toxin

There now appears to be at least four toxins, and probably more, in the ciguatoxin complex, all interrelated and having varying pharmacological actions. Dr. Yasumoto hopes to isolate enough pure ciguatoxin to establish the structure of the complex molecule, possibly in co-operation with Dr. Paul Scheuer of the University of Hawaii.

(c) Pharmacological studies

Dr. Martin Rayner of the University of Hawaii also has been severely limited in his studies of the action of ciguatera on the nervous system, and has not been able to initiate studies on the related toxins because of the lack of adequate amounts of the toxins. Should the toxins become available, he will continue his work.

8. RECOMMENDATION TO WORLD HEALTH ORGANIZATION

To date there exists no nomenclature at the World Health Organization which precisely difines cases of Ciguatera. Yet each year several thousand cases occur in the Indo-Pacific area and the Caribbean Islands. Although the clinical symptoms are very varied they are well known to all medical personnel in endemic areas. The importance of the disease would be better understood if the Health Services would report precise data.

Already the South Pacific Commission has placed Ciguatera on the list of reportable diseases. Unfortunately the rubric employed, E 868, encompasses diverse intoxications, such as toxic berries, poisonous mushrooms, and venemous stings and bites. That is why we propose to the World Health Organization to establish a special designation which could be universally employed. Accordingly, the Committee of Experts recommends that the best way to report Ciguatera would be as:No.988 'Toxic effect of noxious foodstuffs." Within this category we recommend a label exclusively reserved for Ciguatera fish poisoning, as follows :

No.988.3 Oral intoxication by toxic fish.