SOUTH PACIFIC COMMISSION

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EXPERT COMMITTEE MEETING ON CIGUATERA FISH POISONING

(22-26 May 1978, PAPEETE - TAHITI)

Noumea, New Caledonia
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2. INTRODUCTION

The South Pacific Commission has supported and promoted studies and research into ichthyosarcotoxism for numerous years. Fish poisoning is a well-known regional problem of considerable economic and nutritional importance. Gathering information on fish poisoning has recently taken on some degree of urgency as the demand for fishing areas and resources have increased in the South Pacific.

In recent years the Commission has formed a collaborative working group to look into the specific form of fish poisoning known as ciguatera. This group is composed of three independent, but collaborating groups: a Hawaii-based group headed by Dr.A.H. BANNER, a Japan-based group headed by Dr. T. YASUMOTO and a Tahiti-based group headed by Dr. R. BAGNIS. The combined efforts of these three groups have provided the recent significant progress made in ciguatera. The work has been difficult and only within the past few years has the probable source of the toxin responsible for ciguatera poisoning been established. A method for assaying for ciguatoxin has been developed and many of the ecological changes frequently associated with the development of ciguatoxic fish have been determined. The new and continuing developments in this area prompted this meeting on ciguatera.

3. SCIENTIFIC PRESENTATIONS

A portion of the meeting was devoted to the presentation of recent information by the various members of the working group. Each presentation was followed by a discussion to facilitate the exchange of information and to update members on current work. Anyone wishing further information regarding the summarized presentations should contact the author directly; addresses have been provided with the List of Participants.

3.1 ON A CULTURE OF MIXED ALGAE PRODUCING CIGUATOXIN IN HAWAII

Albert H. Banner
University of Hawaii

Hawaii has had, and continues to have, sporadic outbreaks of ciguatera, usually mild, and these have continued. Therefore, after Dr. Yasumoto announced in February 1977 the association of the yet unnamed dinoflagellate (elsewhere referred to as "Diplopsalis")* with ciguatoxin production in the Gambier Islands, and spoke of its association with the alga Turbinaria, we made an initial exploratory survey of Turbinaria on the island of Oahu. No dinoflagellates were found and the search was discontinued.

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* Note: The Diplopsalis sp organisms referred to throughout this document are known to be a new genus and new species. However, the name "Diplopsalis sp" is used here because it is expedient (as suggested by R. Adachi and Y. Fukuyo, Section 3.4).

In early 1978, I found in an unused water-table at the Hawaii Institute of Marine Biology an amorphous grey flocculant material pierced by strands of blue-green alga. Living on the flocculant material was a biconvex thecate dinoflagellate; examination under an ultra-violet microscope showed it carried chlorophyll A. The culture was teeming with the dinoflagellate, with hundreds appearing under the field of a dissecting microscope.

The dinoflagellate was identified by Dr. Max Taylor of the University of British Columbia as the same species that will be named by Drs. R. Adachi and Y. Fukuyo and that Drs. R. Bagnis and T. Yasumoto found in the Gambier Islands. Dr. Y. Hokama of our group at the University of Hawaii found the mixed culture to give a strong positive for ciguatoxin by the radioimmunoassay (RIA) test. We have not yet had confirmation by Dr. Martin D. Rayner's pharmacological test.

There were other plants and animals in the wild culture but it was dominated by a blue-green alga, Microcoleus lyngbyaceus (Lyngbya majuscula), the form that produced the flocculant material. Also present were two species of red algae, a brown alga, diatoms and other protozoans.

We have been successful in subculturing the original mixed culture and some of its component parts, but our results are only preliminary and not quantified, therefore they cannot be reported. It must suffice to report that the mixed culture and its component species have given differing rates of ciguatoxin production, as measured by the RIA.

If this dinoflagellate is found to be unable to produce ciguatoxin in large amounts in axenic culture, then perhaps its relationship to ciguatoxin production (see presentations 3.6 and 3.9) may lie in the mixture of the species in culture. A number of hypotheses suggest themselves as to the reasons for the production of ciguatoxin by the mixed culture. These hypotheses will be evaluated in future investigations.

3.2 STUDIES TO DEVELOP AN ELISA TEST TO ASSAY CIGUATOXIN IN FISH TISSUE

L.R. Berger
University of Hawaii

ELISA (enzyme-linked immunosorbent assay) tests using B-galactosidase, peroxidase and alkaline phosphatase were attempted using fish samples prepared by several methods. Each enzyme and each mode of preparation of the fish sample has certain advantages, some of which will be described in detail. The necessity to use, in parallel, enzyme conjugates of both experimental and normal globulins was demonstrated.

Utilizing anti-ciguatoxin antiserum preparations kindly obtained from Dr. Y. Hokama and phosphatase conjugates, both toxic and non-toxic fish samples were tested by two methods. The first used a hot acetone extraction of small fish samples (0.5g) which were deposited in test tubes or agglutination slides and dried. These fish films served as antigen in the direct ELISA test. The test could be run either qualitatively or quantitatively. The other method used uniform disks of hardened fish tissue each weighing 2mg.

Experiments were performed showing the effect of the direct use of conjugates on: fish samples prepared various ways, samples pre-treated with normal or anti-ciguatoxin globulins, non-toxic fish to which toxin was added in various amounts and by variation of the amount of conjugate used. A sample of toxic dinoflagellates was also tested. The results gave clear indication that there is no detectable difference between normal serum globulins and the anti-ciguatoxin antisera incubated with toxic and non-toxic fish or on the dinoflagellates. The sera react differently on the different specimens, but no difference between the normal and antisera conjugates could be demonstrated.

It is concluded that the sera now available can not be employed for ELISA tests, but that others with greater specificity appear to be necessary, if they can be made.

3.3 RADIOIMMUNOASSAY TESTING OF CLINICALLY DOCUMENTED CIGUATERA POISONING AND SURVEY OF FISHES OF THE LEEWARD ISLANDS OF THE HAWAIIAN CHAIN

Y. Hokama
University of Hawaii

Examination of nineteen outbreaks of ciguatera fish poisoning from fishes obtained from Hawaii, Florida and Midway with the radioimmunoassay using I^{125} labeled sheep anticiguatoxin showed counts per minute per gram (cpm/gm) tissue ranging from 370,597 to 711,346 with a mean of 483,455 ± 79,830. Fishes which showed toxicity included parrot fish, C. rhodochrous, C. cheilio, S. dumerili, and E. mario.

Fishes obtained from the leeward islands of the Hawaiian chain by the National Marine Fishery Service were also examined by RIA. Samples for testing were examined from the following parts of the fish: dorsal, ventral and gonad. Using the following arbitary standards:

1 - Not toxic, less than 350,000 cpm/gm tissue

2 - Borderline " " 351,000 to 399,000 cpm/gm tissue

3 - Toxic, greater than 400,000 cpm/gm tissue

The distribution in per cent for these tissues were as follows:

Tissue Site	Non Toxic	Borderline	Toxic
Dorsal	92.5 %	5.0 %	2.5 %
Ventral	92.0 %	3.2 %	4.8 %
Gonad	67.2 %	12.1 %	20.7 %

The fishes from which the tissues were obtained were not specifically identified.

C. rhodochrous obtained from the Fish and Game Department, State of Hawaii (leeward islands) showed 12 of 14 fishes weighing from 0.2 to 2.2 lbs with cpm/gm tissue greater than 350,000.

3.4 TAXONOMIC STUDIES OF *DIPLOPSALIS sp.*

R. Adachi and Y. Fukuyo
Mie University Kitasato University

Structural studies on the thecal plates of both field and cultured specimens of the toxigenic dinoflagellate from the Gambier Islands have been completed (including the use of scanning electron microscopy). The results of the study have been submitted for publication. We are forced to refrain from giving the new name and the details of the study until the results have been published, but our conclusion is that it is not appropriate to assign this organism to genus Diplopsalis. It is not only a new species, but an organism of a new genus. Interestingly, it is not related to other toxic dinoflagellates such as Pyrodinium sp. and Gonyaulax sp. which are known to cause paralytic shellfish poisoning.

Comment: Until the new name appears in publication, we are obliged to use the tentative name Diplopsalis sp. for the organism.

3.5 AXENIC CULTURE AND GROWTH CONDITIONS OF *DIPLOPSALIS sp.*

A. Inoue
Kagoshima University

The optimum culture conditions for Diplopsalis were sought for temperature, chlorinity, light intensity, and pH. The following various conditions were employed for the tests:

Temperature	:	20°, 27° and 33°C
Chlorinity	:	14.390, 16.378, 19.246, 21.478 and 23.067 %
Light intensity	:	500, 1000 and 2000 lux
pH	:	from 7.2 to 9.0 (at 0.2 intervals)
Test medium	:	Aged sea water enriched with nutrients (ES1 medium)

The results indicated that the highest cell density was attainable by the combination of 19.246% of chlorinity, 27°C temperature and 1000 lux of light intensity. At low light intensity (500 lux), the dinoflagellate showed poor growth and the maximum cell number reached only 200/ml after 30 days of culture. When compared at same chlorinity levels, the maximum growth was attained at 27°C and the intervals for cell division were prolonged at 20° and 33°C. At 27°C, a slight change in chlorinity from 19.246% resulted in a remarkable decrease of cell numbers. The organism could tolerate a rather wide range of pH, but growth at the lowest or highest pH was very poor. The maximum growth was found between pH8.2 and 8.4.

Previous studies carried out by Yasumoto and Bagnis on the wild specimens of Diplopsalis sp. from the Gambier Islands suggested that this dinoflagellate was responsible for the production of both ciguatoxin and maitotoxin. To prove this however, final confirmation should come from an axenic culture. After testing several methods, we succeeded in obtaining an axenic culture. The culture was confirmed to be free of bacteria by employing two test media. The axenic culture will enable us to carry out nutritional requirement studies without the interference of bacteria. Even more important, we can test the effect of organic nutrients on ciguatoxin production, since the organism is suspected of producing ciguatoxin only in the presence of certain organic nutrients.

The bacteria-free specimen was sent to Yasumoto and was confirmed to produce maitotoxin. A portion of the axenic culture was delivered to Yasumoto recently and tests on ciguatoxin production with organic nutrients are being carried out.

3.6 LARGE SCALE CULTURE AND TOXIN PRODUCTION BY *DIPLOPSALIS sp.* BIOCHEMICAL STUDY OF MAITOTOXIN AND CIGUATOXIN

T. Yasumoto
Tohoku University

After trial of many conditions, we have established methods for the large scale culture of Diplopsalis. The ES1 medium was found to be satisfactory to support the growth of this organism. However, the sea water constituent should be taken from less polluted areas and used after treatment with charcoal. The culture should be maintained at 27°C under an illumination of 1000 - 3000 lux and harvested after 5 weeks when the cell density reaches 2000 - 3000 cells/ml. Unlike other free-swimming planktons, use of large tanks is unfavourable for the growth of this organism, probably due to their tendency to rest on the bottom of the container. Neither agitation nor bubbling seems to help their growth. Therefore, our recommendations for large scale culture, at present, is to increase the number of 4-5 litre bottles instead of increasing the size. We now have all the facilities needed for culture and can increase the scale of the culture up to 100 L/month, if funds to assign a person for the maintenance of the cultures are made available.

Several lots of cultured Diplopsalis have been tested for toxin production. The harvested organisms were extracted with boiling methanol and water and the extracts fractionated into diethyl ether, 1-butanol and aqueous fractions according to our routine method. To check for the presence of ciguatoxin, the diethyl-ether fraction was chromatographed on silicic acid and eluted with chloroform-methanol. A mixture (9 : 1) was tested in mice. Only a few fractions yielded a fat soluble toxin which behaved like ciguatoxin on the silicic acid column and the amounts were very small. However, maitotoxin was detectable in every specimen although the yield varied significantly from lot to lot. The production of maitotoxin increased after the culture reached stationary phase. In one experiment, as little as 120 cells were all that were necessary to kill one mouse. The fact that the specimens obtained from axenic culture contained maitotoxin confirms that at least this toxin is a genuine product of the dinoflagellate.

Preliminary experiments were run to test the effect of organic nutrients on toxin production. Extracts from three species of sea weeds, extracts from yeast, and a mixture of glycerol and alanine were added to the culture. Occasionally we could detect toxicity in the ciguatoxin fraction but, again, the amount of toxin was meager. Dense growth of bacteria was observed by the additon of organic nutrients, but the growth of Diplopsalis was remarkably depressed. Hence, we refrain from drawing any conclusion about the effect of organic nutrients until we complete similar experiments with axenic cultures.

Maitotoxin

Purification of the water soluble toxin obtained from Diplopsalis cells was achieved by the use of silicic acid chromatography and gel filtration. Toxin thus purified showed a toxicity of 0.009 µg/g (in mice), which is comparable to toxins such as tetrodotoxin of puffer fish and saxitoxin of paralytic shellfish. When tested for hemolytic activity on mouse erythrocytes, it showed 2.5 times as much activity as commercial saponin. Its toxicity to guppies was 20 times more potent than commercial saponin. Combined with its chromatographic properties, these biological activities indicate that the maitotoxin found in the surgeon fish had actually originated from this organism. Analysis of the purified maitotoxin specimen for amino acid, fatty acid and sugar moieties revealed only small amounts of these moieties in the purified specimen. These moieties previously detected in the maitotoxin specimens obtained from the surgeon fish "maito", might have been derived from contaminants in the sample.

The purified specimen of maitotoxin was sent to the University of Hawaii to be tested for pharmacological properties.

Ciguatoxin

Little work has been done on ciguatoxin due to limited availability of material. We are accumulating, with the kind assistance of Louis Malardé Institute, a stock of toxin for the future experiments. Some attempts are being made to improve the purification procedures by the use of high performance liquid chromatography.

3.7 ECOLOGY OF *DIPLOPSALIS sp.* AND OTHER POTENTIALLY TOXINOGENIC DINOFLAGELLATES : FIELD AND LABORATORY STUDIES*

R. Bagnis
Institut de Recherches Médicales "Louis Malardé"

3.7.1 - Ongoing Study on the Ecology of Potentially Toxinogenic Dinoflagellate Populations

This study deals mainly with Diplopsalis sp.(suspected as being a cause of ciguatera in French Polynesia) and Ostreopsis siamensis (ciguatoxin production unknown). Samplings for these two dinoflagellates were conducted in six locations in Tahiti involving two very different reef ecosystems: (1) the reputedly toxic coastal reef at Hitiaa; and (2) the reputedly non-toxic barrier reef situated at the Papeete passage. The study included weekly sampling of coral and algae, counting of peridinia on Turbinaria ornata, measuring the temperature and salinity of the sea water, in addition to measuring the phosphate, silicate, nitrate, and nitrite content. The observations took place over a period of nearly 18 months. The data collected show enormous variations in the population density of the two dinoflagellates. So far it has been impossible to correlate these density variations to any environmental changes and it would be premature to assume that they are seasonal.

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* In addition to those presenting the papers on research in Tahiti the following people also participated in the research: Dr. J.M. Hurtel, Mr. J.H. Drollet, Mrs. S. Thevenin, Miss M. Garcon, Messrs M. Gay, R. Plichart, C. Hamon, Mrs. F. Moreau, Messrs J. Bennett and G. Jacquet.

At various times of the year in the course of our investigations, we counted the peridinia of the two afore-mentioned species in several locations of Tahiti, Moorea, Hao and the Gambier Islands to get some idea of their distribution in nature. Two major facts emerged from the data collected: (1) presently there is a relatively low density of Diplopsalis in the first three locations compared with the Gambier Islands; (2) the dinoflagellates displayed considerable variation in location and time of their recovery.

3.7.2 - Non-Axenic Cultivation of Potentially Toxinogenic Dinoflagellates and Cyanophyces

We cultured the dinoflagellates Diplopsalis sp., Ostreopsis siamensis, Exuviaella sp., Per. Minusculum and Amphidinium (three species) in addition to the Cyanophycea, Cryptochrysis sp., Isochrysis sp and Chroococcopsis. The organisms were isolated from fresh samples or from laboratory cultures maintained under strong or weak light. The culture medium consisted of sea water enriched with Provasoli ES_1 supplement. The temperature was maintained at between 25° to 29°C.

The most interesting findings were obtained with the cultures of Diplopsalis sp. We were able to differentiate two types of cells. The first type (75 µ) had an irregular round shape and a slow irregular growth pattern. The second cell type (55 µ) was more regular in shape and grew rapidly, especially in the presence of bacteria. The existence of a stable population of bacteria enabled us to regulate the growth of this cell type. We were not able to prove the synergistic action of Diplopsalis with the bacteria, but certain observations suggested that the peridinia was capable of absorbing organic substances bypassing the need for photosynthesis.

3.8 CIGUATOXICITY OF CORAL AND ALGAE. RELATIONSHIP BETWEEN THE TOXICITY OF *CTENOCHAETUS STRIATUS* AND POPULATIONS OF *DIPLOPSALIS* AND *OSTREOPSIS SIAMENSIS*

Suzanne Chanteau
Institut de Recherches Médicales "Louis Malardé"

3.8.1 - Distribution of Ciguatoxicity in Samples of Coral and Algae

The biological residues obtained from scraping coral and washing red calcarious algae obtained from the Gambier Islands were sifted through screens ranging in size from 500 µ to 40 µ. After the Diplopsalis had been counted, the various fractions obtained by this screening were extracted with boiling methanol. A correlation between ciguatoxicity (ciguatoxin and maitotoxin) and the number of Diplopsalis was not always present and varied from sample to sample. Maximum toxicity was obtained sometimes in the 500 µ and 400 µ fractions where only few Diplopsalis were shown to be present. Insufficient scraping, the presence of Diplopsalis debris and the age of the Diplopsalis could not entirely account for the discrepancy.

There is little doubt that ciguatoxin is produced by Diplopsalis because it has been demonstrated in vitro, but is not at all certain that this dinoflagellate is the only one responsible for ciguatoxin production.

From the coral specimens collected in Tahiti, where very few Diplopsalis but many Ostreopsis siamensis were found, ciguatoxin was absent.

3.8.2 - Relationship between the Toxicity of Ctenochaetus striatus and Populations of Diplopsalis and Ostreopsis siamensis

Populations of Diplopsalis and Ostreopsis siamensis were counted weekly in the locations of Hitiaa and Papeete. Concurrently, the toxicity of the flesh, liver and viscera of C. striatus was tested every month over a period of one year. Diplopsalis had virtually disappeared from the reef since April 1977; from March 1977 onwards the toxicity of C. striatus flesh and viscera did appreciably diminish, however there was considerable fluctuation in liver toxicity which was difficult to explain.

3.9 PREPARATION OF PURIFIED CIGUATERA TOXINS. TOXICOLOGICAL AND BIOCHEMICAL STUDY ON THE EXTRACTS OF VARIOUS CULTURED ALGAE

Eliane Chungue
Institut de Recherches Médicales "Louis Malardé"

3.9.1 - Purification of Various Toxins

Several lots of scaritoxin, ciguatoxin and maitotoxin were obtained respectively from parrot fish, moray eels, groupers, snappers and from wild Diplopsalis cultures. The purified toxin was used for studies carried out at the University of Hawaii and the Institut Malardé. A certain quantity of these purified extracts is still available for research purposes.

3.9.2 - Toxicological and Biochemical Studies on the Extracts of Various Cultured Algae

Fifteen monoalgae cultures, 4 of Diplopsalis, 9 of other dinoflagellates and 2 of Cyanophyceas were filtered and the residue extracted with boiling methanol. The majority of the extracts proved non-toxic or only slightly toxic except for cultures of Chroococcopsis sp.II, Exuvisella sp. and Diplopsalis.

We studied the chromatographic behaviour of the acetone-precipitable water soluble and fat soluble fractions of Amphidinium I (No.1), Diplopsalis (Nos. 11, 13 and 14) and Exuvisella (No.15) cultures. For the water soluble acetone-precipitable fractions of the various toxic algae cultures the chromatographic behaviour was the same as maitotoxin. Thus this toxin appears to be common to various algae.

Only the fat soluble fraction from Diplopsalis No.14 contained toxins of the ciguatoxic type. One was identical with scaritoxin, both in chromatographic behaviour and the symptoms it produced in mice. The other was similar with ciguatoxin in its effect on mice, but slightly different in its chromatographic behaviour. Like scaritoxin, it was eluated by chloriform from a DEAE column, but the Rf. values of the "toxic band" were between 0.28 and 0.46, whereas those for scaritoxin are between 0.5 and 0.7 and those for ciguatoxin are between 0 and 0.3 under the same experimental conditions.

These results are comparable to those obtained from some of the biological matter deposited on coral. They also point out the problem of establishing chemical relationships between ciguatoxin and other toxins of the ciguatoxic type.

3.10 PRELIMINARY STUDIES ON AN IMMUNOLOGICAL TEST FOR DETECTING CIGUATOXIN IN SAMPLES OF FISH TISSUE

F. Parc
Institut de Recherches Médicales "Louis Malardé"

We conducted a correlative study on the same samples between the cat toxicity test performed at the Institut de Recherches Médicales "Louis Malardé" and the radioimmunoassay (RIA) using I^{125} labeled sheep anticiguatoxin (kindly supplied by Dr. Y. Hokama, University of Hawaii). Seventy-seven tissue samples of various toxic and non-toxic fish were tested. The results suggested that the immune sera of the RIA may lack some specificity in detecting toxic tissues. The tests were also performed on homogeneous lots of toxic parrot fish and moray eels. The RIA did not show any significant correlation with the degree of toxicity. Because of its insensitivity at this stage, if the RIA were used to detect ciguatoxic fish, a minimum of 80 per cent of edible species would be eliminated.

The same sheep anti-ciguatoxin immune serum was tagged with fluorescene for use in the fluorescent antibody test; as controls, normal sheep, mouse and hen sera were also tagged with fluorescene. No specific fluoresence was observed with the ciguatoxin-producing dinoflagellates. The fish tissues tested showed an ability to non-specifically adsorb various amounts of immunoglobulin. The degree of adsorption varied with the fish species and the organ tested, but there did not appear to be any relationship to the presence of ciguatoxin.

Tests were also carried out on the ability of purified ciguatoxin to attach to various serum proteins. Purified ciguatoxin was mixed with normal and immune rabbit sera; it was not possible to distinguish between what was non-specific biochemical binding and what should have been an antigen-antibody reaction.

The general conclusions of these studies are as follows:

1. The present anti-ciguatera antisera cannot be used in the radioimmunoassay in French Polynesia until the specificity has been improved.
2. Development of an immunologic test for ciguatoxin may be difficult, if not impossible, because of the complex interactions occurring between the fish tissues and gammaglobulins on one hand, and the ciguatoxin and proteins on the other.

3.11 CLINICAL OBSERVATIONS ON 3009 CASES OF CIGUATERA FISH POISONING IN THE SOUTH PACIFIC

T. Kuberski
South Pacific Commission

Relatively little detailed clinical information is available on ciguatera fish poisoning and only a few accounts on the more overt cases have been published on this topic. Between 1968 and 1976 the clinical findings were recorded on 3009 cases of presumed ciguatera fish poisoning from several South Pacific islands where this disease is a particular problem. This paper describes the clinical findings in these 3009 patients.

Distribution of cases by age group and sex

Fifty-nine per cent of the patients were males, forty-one per cent females (male/female ratio of 1.4/1). The disease was not significantly more prevalent in either sex in any of the age groups. Almost fifty per cent of the cases were in their 2nd or 3rd decade.

Onset of illness

Seventy-seven per cent of patients had the onset of their symptoms less than 12 hours after ingestion of a presumed toxic fish. Almost all (96%) of the patients developed symptoms by 24 hours after ingestion.

Clinical symptoms

The most common presenting complaint of these patients was paresthesias. These were either numbness or tingling in the extremities (88.2%), circumoral paresthesias (88.1%), or a painful sensation on the skin when in contact with cold water (86.5%). Paresthesias were common, but arthralgias and muscle aches were almost as equally common. Gastro-intestinal symptoms of diarrhoea, nausea and vomiting were relatively frequent. Weakness, vertigo and ataxia were also common complaints. Chills and headache, without fever, were also observed. Pruritis was seen in forty-four per cent of patients.

Duration of illness

About one third (33.6%) of the patients were required to go to bed because of their illness. Over 60% stayed in bed for only one or two days. However, a small proportion (6.4%) did require a week or more in bed as a result of their illness.

Physical signs

Pupil size, reflexes and temperature were generally recorded as normal (> 95%). General physical examination was not contributory unless the patient was severely ill. Examination to determine the boundaries of the paresthetic lesions found them to be ill-defined and to follow no apparent distribution. Hypotension was recorded as being present in about ten per cent of patients. In those patients in whom a systolic blood pressure measurement was actually made, 14% were observed to have a systolic blood pressure of 100 mmHg or less. Measurement of the pulse rate disclosed a bradycardia (rate < 60/minute) in over 13% of patients. There was no difference in these physical findings between males and females.

Discussion

This study is not entirely satisfactory in that no diagnostic test exists to confirm patients as having ciguatera fish poisoning. This may therefore bias the information towards those symptoms thought to be associated with ciguatera fish poisoning. Paresthesias are considered the clinical hall-mark of ciguatera and therefore might account for the preponderance of this finding amongst these patients. Were paresthesias not present, this type of fish poisoning may well be extremely difficult to differentiate from other forms of food poisoning or mild forms of infectious gastroenteritis. A definite description of signs and symptoms needs to await the development of a way of definitively detecting disease due to ciguatera fish.

(This information was compiled and collected in collaboration with Dr R. Bagnis and others at the Institute of Medical Research "Louis Malardé" Tahiti and Ms S. Laugier).

4. RECOMMENDATIONS TO THE SOUTH PACIFIC COMMISSION REGARDING THE CIGUATERA FISH POISONING PROJECT

Following discussions on the recent advances made by the three working groups, the Expert Committee members formulated recommendations to the Commission for providing support for further investigations into the problem of ciguatera fish poisoning.

4.1 Immediate priorities

4.1.1 - Development of a Pure Culture of Ciguatoxin-producing Dinoflagellates

This priority will be pursued by the Japanese team (Drs. Yasumoto and Inoue) and at the University of Hawaii (Dr. Banner). The Japanese team will be developing axenic cultures of ciguatera-producing dinoflagellates so that pure cultures of these organisms can be used for point 4.1.2.

Dr. Banner will look into the recent finding of a dinoflagellate-blue-green alga culture which is apparently producing ciguatoxin. The cause of ciguatoxin production by this culture needs to be evaluated thoroughly.

4.1.2 - Large scale Production of Ciguatera-producing Dinoflagellates

Fundamental studies on ciguatoxin have been greatly hampered by the lack of pure ciguatoxin in quantity. Mass culture techniques may allow the recovery of toxin in quantity. This technique should greatly reduce the expense and difficulty of the biochemical extraction of toxic fish. A highly pure ciguatoxin is needed for:

(a) Determining the chemical structure of ciguatoxin.

Without pure ciguatoxin, determining the chemical structure will be extremely difficult. The structure is believed to be primarily lipid and complex, elucidation of its structure will require sophisticated biochemical techniques. This work will be initiated by Dr. Yasumoto's group.

(b) Determining the pharmacological action of ciguatoxin.

The pharmacological action of ciguatoxin has not been determined in detail and is needed for developing improved detection methods and rational approaches to therapy. Dr. J. Miyahara (University of Hawaii) should be contacted regarding his potential collaboration in this project.

(c) Development of practical and precise immunological assays for ciguatoxin.

The availability of a pure toxin may allow the development of highly sensitive and specific assays for ciguatoxin. Immunologic tests appear to be the most applicable to this situation and production of quality antiserum is paramount. Evaluation of various sources and methods of preparation of antisera to ciguatoxin should be explored (Dr. Parc).

4.1.3 - Study on the Ecological Requirements of Diplopsalis

The growth requirements of Diplopsalis in the laboratory and in nature is currently being done and needs continuing support. This information is important for the laboratory growth of Diplopsalis in culture, and also for determining the ecological factors which promote the growth of, and toxin production by, Diplopsalis in nature and subsequent fish toxicity.

Laboratory findings must be correlated with field studies and it is considered a priority that Dr. Bagnis' group continue their studies on the ecology of reefs and its relationship to ciguatera.

4.2 Future and Continuing Research

4.2.1 - Characterize the Human Immunologic Response to Ciguatera Poisoning

Patients suffering from ciguatera multiple times frequently develop what appears to be a hypersensitivity to fish. The immunological response developed by these patients needs to be examined to determine rational approaches to treatment.

4.2.2 - Evaluation of the Treatment in Ciguatera

No known cure for ciguatera exists and only empirical modes of therapy are available. A systematic evaluation of the rational treatment of poisoned patients needs to be carried out.

4.2.3 - Blood Chemistry Analysis in Cats with Ciguatera

Possible changes in blood chemistry during ciguatera fish poisoning may provide a clue to the physician that ciguatera is present. A systematic study of blood chemistries in cats known to be poisoned by ciguatera under controlled conditions may provide answers. Serial bleedings of known poisoned animals can also provide sera for circulating ciguatoxin analysis and the development of antibody.

4.2.4 - The South Pacific Commission is Encouraged to Seek Outside Funding for the Ciguatera Project

The recent exciting developments in the understanding of ciguatera fish poisoning has opened doors for further significant advances. The Commission has supported this project for many years and the current findings need further investigation which would be difficult to fund within the Commission's budget. The working group on ciguatera suggests that, through the Commission, funding from international agencies might be obtained in light of the international team effort attempting to resolve the regionally important problem of ciguatera.

4.2.5 - Meetings of the Ciguatera Working Group

A meeting of the Commission's Expert Committee should meet every other year to exchange information and assist the Commission in determining priorities for their special project on fish poisoning.