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Laboratory of Biochemistry, Faculty of Medicine and Pharmacy of Casablanca, Morocco

## **Chick feeding test: a simple system to detect ciguatoxin**

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### **Summary**

Chick poisoning induced by oral administration of toxic fish tissues or extracts gave rise to internal hypersalivation, decrease in weight and acute motor ataxia. Detoxification was low and repeated administration therefore led to toxin accumulation. Response of the chicken to liver feeding was roughly quantitative; so liver, which is the most potential toxic tissue, may be used for a preventive screening test in ciguatera-endemic areas.

**Key words:** ciguatera; chick bioassay; feeding test; Caribbean.

### **Introduction**

Ciguatera poisoning is a tropical disease caused by the ingestion of a wide variety of coral reef fishes (Halstead, 1978). The main causative toxin, ciguatoxin, has been isolated in the Pacific (Scheuer et al., 1967; Nukina et al., 1984) and it is present in the Caribbean (Vernoux et al., 1982). To determine which fish are safe for consumption, feeding or injection tests on sensitive animals are still commonly used (Hoffman et al., 1983; Hokama et al., 1983; Chungue et al., 1984). As most ciguateric areas are small islands without facilities, feeding tests remain the method of choice for an everyday preventive use since it needs no particular equipment. However, the usual test animals, cats (Bagnis and Fevai, 1971) and mongooses (Banner et al., 1960), are not quite satisfactory since the former often regurgitates part of the toxic flesh and the latter is usually infected with several diseases (Banner, 1976) and not everywhere available. Furthermore both animals, which must be fed 10% of their body weight, are comparatively large, thus prohibiting the screening of small fish (Scheuer, 1977). Our laboratory has therefore looked for another test animal which is smaller, easy to

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handle and available everywhere. The chicken was selected as a possible candidate. The present study deals with ciguatera poisoning of the chicken after oral administration of toxic fish tissues or extracts and its use in Saint Barthelemy island, a ciguatera-endemic area (Bagnis, 1979).

## Material and Methods

### *Source of ciguatoxin*

Ciguatoxic fishes (*Caranx bartholomaei*, *Caranx latus*, *Caranx ruber*, *Seriola rivoliana*, *Seriola dumerili*, *Gymnothorax funebris*, *Gymnothorax moringa*, *Mycteroperca venenosa*, *Epinephelus morio*, *Sphyaena barracuda* and *Scomberomorus cavalla*) were collected around Saint Barthelemy (a small island of the French Caribbean) and kept frozen until use. Liver was removed from the viscera; one part was used in the feeding test and the other was extracted (and the homologous flesh as well) by our routine acetone method (Vernoux et al., 1985) to prepare toxic lipid-soluble residue. Toxin concentration (TCC) in tissue was calculated from the MLD (minimum lethal dose) in mice as already described (Vernoux et al., 1985). TCC is the amount of toxin in 1 g of the original tissue and is expressed in mouse units per gram of tissue (MU/g). (1 MU is defined as the minimum amount of toxin required to kill 1 g of mouse within 24 h.)

### *Feeding tests*

White Arbor Acres chickens (Cicalim, Casablanca), weighing 70–100 g and 8–10 days old were force-fed with 10% of their body weight of minced and homogenized cooked tissue.

To screen individual fish for ciguatoxicity at Saint Barthelemy, liver was cooked for 15 min in a sealed bag (“Seal a meal”, boilable cooking pouches Dazey products Co), then manually homogenized and the chicken (70 g or more<sup>1</sup>) was force-fed (10% of its body weight) by pushing the liver mixture in the crop through a tube (length: 10 cm; internal diameter: 2.5 mm) fitted on a 50 ml disposable syringe. Response to liver feeding was checked after a 48-h period. A simple way to distinguish between negative and positive results is to weigh the chicken before and after the 48-h period: negative results correspond to an increase in weight (>20% at least). People with practice may read the results after but 24 h.

## Results

The presence of ciguatoxin was demonstrated in the lipid-soluble extracts by chromatographic studies (Lahlou, unpublished results). Furthermore, characteristic ciguatera symptoms, as described elsewhere (Vernoux et al., 1985), were checked after i.p. injection of the same extracts in the mouse.

### *Oral poisoning of the chicken*

Feeding of liver, spleen, ovaries, kidney, flesh or lipid-soluble extracts from toxic fish elicited ciguatera symptoms in the chicken (the first symptoms always occurred within a 24-h period). The progression of symptoms was graded as indicated in Table 1. No regurgitation or diarrhea was observed and death occurred after 1 to 7 days, depending on the dose. Sublethal dosage includes always grade +1 poisoning over a 48-h period.

<sup>1</sup> The size of the liver sample (= SL), which is only 1–2% of fish body weight, determines the maximum permissible size of the chicken (= 10 SL).

Table 1. Chicken assay: ciguatoxin ratings

Toxicity rating	Response observed in chicken
0	No visible response
+1	Loss of vivacity, frequent mastication, head shake, heavy eye-lids, arrested growth
+2	Loss of escape reflex, refusal of solid food, dilatation of the crop*, continual standing up
+3	Beginning of motor ataxia (wobbly gait but ability to stand up), refusal of water
+4	Total and irreversible inability to stand up due to acute motor ataxia, dyspnea
+5	Death

\* due to internal hypersalivation

### *Ciguatoxin accumulation*

Repeated administration, once a day, of toxic extracts at a subsymptomatic level, induced lethality; nevertheless for the same cumulative dosage, lethality decreased with the number of feedings (Fig. 1). Toxin accumulation therefore occurred in the chicken concurrently with detoxification. When a single sublethal dosage elicited a +1 response 48 h after oral feeding, the subsequent feeding of extract at the same dose level to the same chicken had to be retarded by at least 7 days in order to have no lethal effect, i.e. no toxin accumulation. This showed that detoxification is low.

### *Assay of fish liver in chick feeding experiments*

Response of chicken to liver feeding, monitored over a 48-h period, was roughly quantitative (Fig. 2). Liver TCC from 1.5 to 2 MU/g elicited a grade +1 response, 2 to 5 MU/g corresponded to grade +2 response, and grade +3, +4 and +5 required higher dosage. A clinical response was therefore obtained when one gram of the assayed fish liver contained at least 1.5 MU. This limit may be lowered by feeding the animals repeatedly, once a day. In all experiments the toxin concentration of the flesh was lower than that of corresponding liver sample (Fig. 2) and ratios of liver to flesh TCC were  $>3$ .

### *Screening fish for ciguatoxicity by the chick feeding test*

As liver is a more toxic tissue than flesh it has been used for the screening test at Saint Barthelemy since 1980 (Vernoux, 1981). The positive or negative results obtained over a 48-h period were extrapolated identically to the corresponding flesh. In this way we were able to select only toxic as opposed to non-toxic specimens for research purposes. Furthermore, when negative results

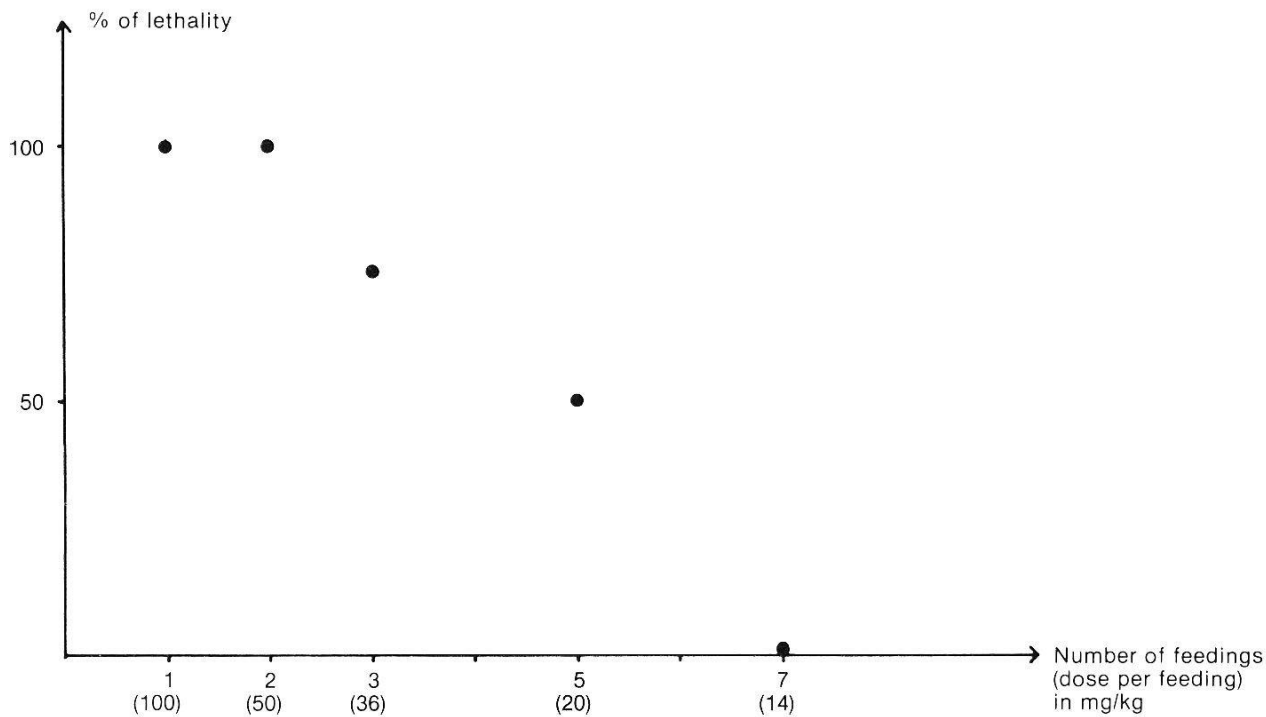


Fig. 1. Ciguatoxin accumulation in chicken: dependence of lethality on the number of feedings of a single cumulative dosage (100 mg/kg given once a day to 4 animals per dose).

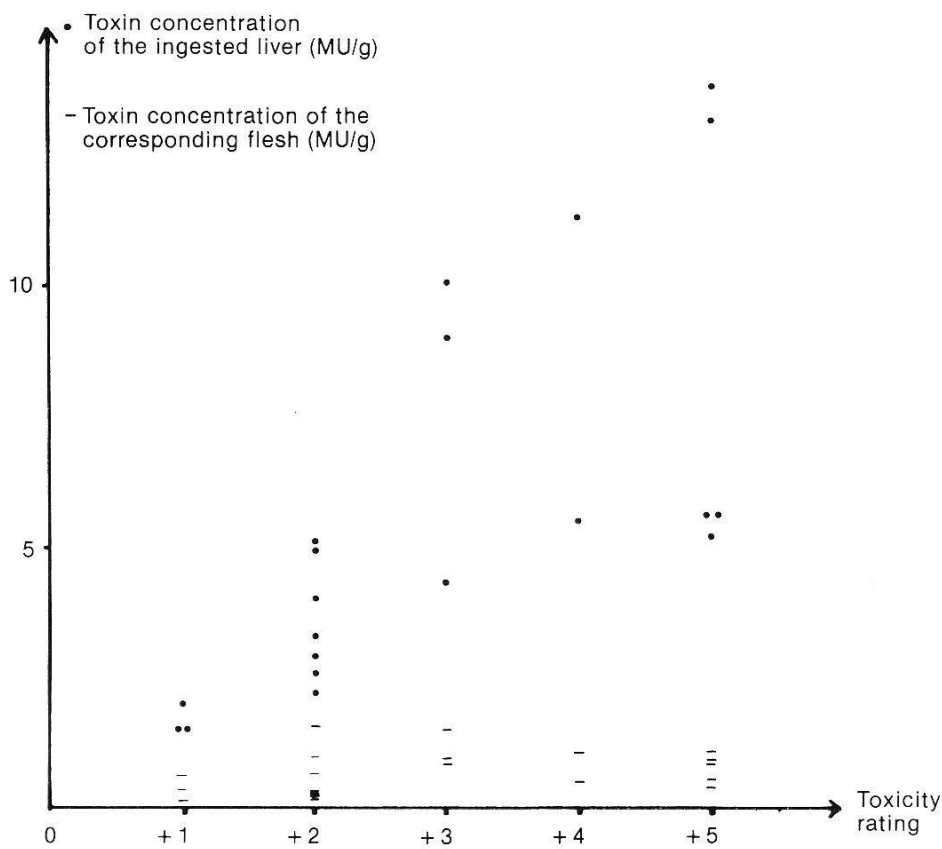


Fig. 2. Chick assay of ciguatoxic liver: relation between toxin concentration of various livers and toxicity rating over the 48-h test period.

occurred the fish was considered to be safe for consumption, although low toxin concentration ( $<0.5$  MU/g which is the threshold concentration of clinical poisoning according to Bagnis, 1981) was sometimes detected. Such fishes were consumed by one of us (J. P. Vernoux) without any problem.

## Discussion and Conclusion

In ciguatera-endemic areas in the French West Indies the hen has been used to assay fish for ciguatoxicity (Ebroin, 1972). Li (1970) reported demyelination in both spinal cord and sciatic nerve in the adult hen after i.m. ciguatera poisoning. Kosaki et al. (1968) showed that the chicken (white leghorn) is as satisfactory as the mouse for i.v. or i.p. bioassay. However, Banner et al. (1960) used chicks for checking ciguatoxin by voluntary feeding on ground-dried flesh and obtained negative results, though the authors pointed out that there was no assurance that the chicks ingested sufficient quantities of toxic fish to cause a reaction. The negative results of Larson and Rivas (1965) may be explained the same way, as the ingested quantity of toxic homogenate per gram of chicken was well below that used to elicit ciguatera symptoms in cats. As our chickens were younger than those used by Banner et al. (1960) or Larson and Rivas (1965) we thought that sensitivity might vary according to age. Since ciguatoxin is a neurotoxin with central effects (Legrand et al., 1982) we suggested that the blood brain barrier which develops in chickens 28–30 days old could play a role in ciguatera poisoning. Preliminary results, however, using chickens older than 30 days, did not confirm this hypothesis.

In the animal sensitivity scale to ciguatoxin the chicken is close to the cat since, according to Bagnis and Vernoux (1976), ciguatera poisoning was induced in cats after ingestion of a meal of toxic flesh (10% of its body weight) containing at least 1.4 MU/g of flesh (lethal effects were constant above 3 MU/g of flesh and toxin accumulation was also demonstrated). In the present study we obtained very similar responses of the chicken to liver feeding. For feeding experiments it is advisable to use the chicken rather than the cat because the former does not regurgitate and is both easy to handle and to obtain. Its small size allows a preventive screening test for fish ( $\geq 0.5$  kg) based only on the feeding of liver, i.e. the most toxic tissue (Vernoux et al., 1985). This simple test system could be applied by the local population on small islands without scientific equipment and it is sufficiently short (48 h at the most) to allow the keeping of the fish until results are known. This chick test remains wholly valid until immunology can bring us a simpler solution even though the formation of spontaneous ciguatoxin-protein complexes (Parc et al., 1979; Emerson et al., 1983) clearly complicate the search for antibody specific for ciguatoxin.

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