BULLETIN OF THE BINGHAM OCEANOGRAPHIC COLLECTION

The *Bulletin of the Bingham Oceanographic Collection,* established by Harry Payne Bingham (Yale 1910) in 1927, published scientific articles and monographs on marine and freshwater organisms and oceanography for the Bingham Oceanographic Collection at Yale University.

The series ceased independent publication after Volume 19, Article 2, and was merged into the *Bulletin of the Peabody Museum of Natural History* monograph series after 1967.

See also the Bingham Oceanographic Collection Archives,
Invertebrate Zoology, Yale Peabody Museum, in the Archives at Yale:
https://archives.yale.edu/repositories/15/resources/11140

Yale PEABODY MUSEUM OF NATURAL HISTORY

P.O. Box 208118 | New Haven CT 06520-8118 USA | peabody.yale.edu

BULLETIN

OF

THE BINGHAM OCEANOGRAPHIC COLLECTION

PEABODY MUSEUM OF NATURAL HISTORY
YALE UNIVERSITY

VOL. IX, ART. 1

TETRODON POISONING

BY WARREN H. YUDKIN

Bingham Oceanographic Laboratory

Issued February, 1944

New Haven, Conn., U. S. A.

PUBLISHED BY

BINGHAM OCEANOGRAPHIC LABORATORY

"Founded for the Purpose
of Oceanographic Research"

TETRODON POISONING

By Warren Harold Yudkin*

Bingham Oceanographic Laboratory, Yale University

TABLE OF CONTENTS

	Page
Abstract	1
Introduction	2
History and Distribution	4
Toxicity and Chemistry	10
Action and Symptoms	13
Discussion	16
References	17

ABSTRACT

The common puffer, *Spheroides maculatus*, has recently attained market status as a result of the increased utilization of "trash" fish. However, it belongs to the Tetraodontidae, many of whose members are notably toxic. This paper summarizes the pertinent information on tetrodon poisoning (History and Distribution, Toxicity and Chemistry, Action and Symptoms, etc.); in so doing it provides evidence that the toxic substances of many Tetraodontidae are located chiefly in the gonads and liver and may vary quantitatively with the degree of maturation. It also indicates the expediency of seasonal and other toxicity tests on *S. maculatus* and of special care in its preparation for market until it has been more thoroughly investigated.

* The Connecticut State Board of Fisheries and Game, cooperating with the Bingham Oceanographic Laboratory in its study on the utilization of marine resources, generously provided funds, a part of which has been used in the present investigation.

The writer is grateful to H. George Mandel and Frank H. Sommer III for their assistance in translating pertinent German and Spanish articles, as well as to Martin D. Burkenroad and Grace Pickford for their careful reading of the manuscript. He is also indebted to Daniel Merriman, not only for the introduction and discussion which he has written, but also for the suggestions and constructive criticisms given throughout the formation and writing of this paper.

INTRODUCTION

During the past year the efforts of a number of individuals and organizations have been directed toward the increased utilization of marine resources. That work along these lines should be intensified under emergency conditions is in no way surprising. On the other hand, it is an unfortunate commentary that there should be such a multiplicity of unsolved problems in this field. Considering only the species which are discarded from the commercial catch as "trash," but which are in reality potential sources of food for human or other consumption, it is common knowledge that the wastage is of great magnitude. Yet adequate figures on seasonal composition in many different fisheries are not available. And the inadequacy of the available information on "trash" fish is further evidenced by the fact that there is not even unanimity of opinion as to which of these forms should be utilized.

Among the "trash" species which have been recommended lately as food fish is the common puffer, *Spheroides maculatus* (Bloch and Schneider), of the Atlantic coast. This fish is abundant and is taken in large quantities in certain seasons by trap-net and other fisheries. It has been eaten in various local communities for many years, and is generally recommended as having an exceptionally good flavor. The fish has not had market status until recently, mainly because there was an insufficient demand for it and also because the percentage of the total weight that can be recovered as the edible portion is relatively small. But at the present time the puffer appears on the market in limited quantities, both fresh and frozen.

However, *Spheroides maculatus* belongs to the family Tetraodontidae, many of whose members are notably toxic.[1] Thus a number of

[1] Jordan (1925) states that "poisonous alkaloids are developed in the flesh" throughout the Plectognaths as a whole. Among those specifically mentioned as being toxic are some of the Balistids, Ostraciids, Tetraodontids, and Diodontids. The members of the latter two families have many popular names, a number of which are common to individuals in both groups. This fact, in conjunction with the wide variation in scientific terminology, leads to frequent confusion. Some of the names applied to the Tetraodontidae and their close relatives follow: swellfish, swellbelly, swelltoad, blowfish, pufferfish, globe fish, porcupine fish, balloon fish, toadfish, blaser (in the Dutch East Indies), fugu (in Japan), Ikan buntal (in Malaya), tambores (in Cuba), botete (in the Philippines), etc. In the present paper "tetrodon" is used in a general sense, except where authors quoted do otherwise or in dealing with those references in which the specific identity of the fish involved is clear.

people die of tetrodon poisoning each year in Japan. For example, Nagai and Ito (1939) say, "The ovary of the Fugu fish (Spheroides) contains a strong poison, about 20 people a year becoming its victims." The poisoning also assumes serious aspects in the West Indies, East Indies, Malaya, the Philippine Islands, and the Cape of Good Hope, according to various authors. In view of the close relationship of *S. maculatus* to these toxic species, it seems appropriate to examine the literature on tetrodon poisoning for evidence as to whether or not special care need be exercised in utilization of this form, and also as to the advisability of testing it for possible toxic effects.

That this fish has been eaten for some time in various regions with few if any definite reports of ill effects is a clear indication that its flesh is not ordinarily toxic. This does not prove, however, that it might not be poisonous under certain circumstances. In this connection several possibilities immediately present themselves. Thus it is not inconceivable that Dinoflagellates, only occasionally appearing in sufficient abundance to be effective, might impart toxic qualities to the flesh (Nigrelli, 1936). Much more probably, a toxic substance might be located in the viscera and might result in poisoning only when the fish are improperly cleaned, or when they remain ungutted for some time after their capture so that the poison has an opportunity to permeate the muscle. With regard to the latter alternative, there is also the possibility that the toxic substance might be seasonal in its appearance or concentration—*i. e.*, it might, for example, be asssociated with the maturation of the gonads and therefore would manifest itself only before and during the spawning time. It is hoped that the summary of the available information on tetrodon poisoning which is presented here will indicate the need for research which will result in one of the following alternatives: the complete vindication of *Spheroides maculatus,* recommendations for special care in its preparation for market sale, or its condemnation as a species which can be utilized with reasonable safety. Such research is at present in progress in the Bingham Oceanographic Laboratory, but should unquestionably be undertaken by other workers in different localities if the answers to this problem are to be in any way complete.—D. M.

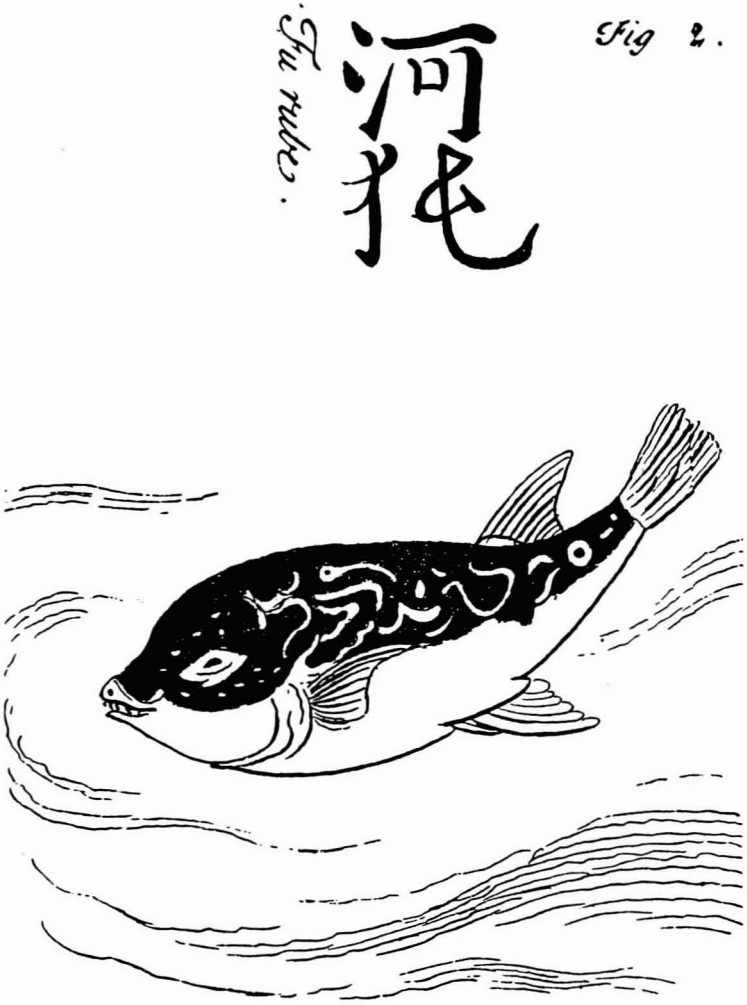
HISTORY AND DISTRIBUTION

There is record of the poisonous qualities of the blowfish of the family Tetraodontidae as early as 1690, for Engelbert Kaempfer, in his exhaustive history of Japan describes in some detail the early Japanese customs surrounding the eating of the fish. Evidently it had considerable notoriety throughout the islands.

> "Furube is another Fish, not very large. The Dutch call him Blazer, which signifys Blower, because he can blow and swell himself up into the form of a round Ball. He is rank'd among the poisonous Fish, and if eat whole, is said unavoidably to occasion death . . . This the Japanese reckon a very delicate Fish, and they are very fond of it. But the Head, Guts, bones, and all the garbage must be thrown away, and the Flesh carefully wash'd and clean'd before it is fit to eat. And yet many people die of it, for want, as they say, of thoroughly washing and cleaning it . . . And yet the Japanese won't deprive themselves of a dish so delicate in their opinion, for all they have so many Instances, of how fatal and dangerous a consequence it is to eat it."

Thus, from early times the tetrodon was considered poisonous by the Japanese, yet the important fact remains that the fish was eaten at least as early as the 17th century, provided all the viscera were removed and the remaining flesh carefully washed. Kaempfer also states that "it is sold much dearer than common Fish, and not eat, but when fresh." This suggests that the toxic qualities of the tetrodon lie in the viscera, and that the fish must be carefully washed to remove any poison left after the fish has been eviscerated. It also leads to the suspicion that the fish cannot be allowed to remain ungutted too long before being eaten, presumably because the poison from the viscera permeates the tasty skeletal muscle.

Kaempfer further informs us that, "Soldiers only and military men, are by special command of the Emperor forbid to eat this fish. If any one dies of it, his son forfeits the succession to his father's post, which otherwise he would have been entitled to." The above rules were made in order to decrease the number of suicides that were accomplished by eating "Furube," or "Fugu," as the Nipponese now call it. This is further emphasized by mention of another kind of tetrodon, different from that previously mentioned because "the poison of this sort is absolutely mortal, no washing nor cleaning will take it off. It is therefore never ask'd for, but by those who intend to make away with themselves."



The fugu (or furube) as pictured in Kaempfer's History of Japan, 1728. This is probably the earliest representation of this toxic fish to appear in occidental literature.

Osbeck makes mention of the toxic qualities of the tetrodon as the Chinese know it in his *Voyage to China and the East Indies* in 1771:

> "Kay-po-oy, *Diodon ocellatus* (*Tetrodon ocellatus* Linn.) is one of the finest fish I ever saw, but so poisonous that whoever eats of it generally dies in two hours time. The Chinese, who affirmed the fact, seeing me take the fish into my hands, earnestly desired me to wash myself, adding that it is forbidden under some great penalty, to be sold among other fish."

He then goes on to describe the fish in detail and states that it was taken at Canton and a "civil sailor, who was present when the Chinese caught it, gave it to me."

Tetrodon poisoning appears in the literature again several years later (Cook, 1777; Forster, 1777 and 1778). On Wednesday afternoon, September 7, 1774, when Captain Cook was at New Caledonia in the progress of his second voyage around the world, his clerk purchased a tetrodon from a native fisherman. Since the fish proved to be one never seen before, it was drawn and described. This process took so long, however, that only the liver and the roe were dressed for supper, which organs Captain Cook, his naturalist George Forster, and the elder John Forster, merely tasted. Then, according to Cook's description:

> "About three o'clock in the morning, we found ourselves seized with an extraordinary weakness and numbness all over our limbs. I had almost lost the sense of feeling; nor could I distinguish between light and heavy bodies, of such as I had strength to move; a quart pot, full of water, and a feather, being the same in my hand. We each of us took an emetic, and after that a sweat, which gave us much relief. In the morning, one of the pigs, which had eaten the entrails, was found dead. When the natives came on board and saw the fish hang up, they immediately gave us to understand it was not wholesome food, and expressed the utmost abhorrence of it; though no one was observed to do this when the fish was to be sold, or even after it was purchased."

George Forster's journal gives a similar story and in addition assigns the fish to the genus *Tetrodon:*

> "It was of the genus, by Linnaeus named *tetraodon*, of which several species are reckoned poisonous. We hinted this circumstance to captain Cook, especially as the ugly shape, and large head of the fish, were greatly in its disfavor; but he told us he had eaten this identical sort of fish on the coast of New Holland, during his former voyage, without the least bad consequences. At supper

the liver of this fish was served up, which was very large and oily. For this reason the captain, my father, and myself eat only a morsel or two of it, and thought it had no other bad taste, than what arose from the oil."

The description of the effects of the poison is much the same as that given by Cook but goes into more detail:

" . . . the blood had left our cheeks, all our limbs were benumbed, and without sensation, and a great degree of languor and oppression had taken place. Emetics were administered, which gave my father and myself some relief, but had not much effect upon captain Cook. We took a sudorific after this, and went to bed again . . . The poison which had been thus fatal to us, had likewise affected several dogs, taken on board at the Society Islands; these creatures having seized upon the remains of the liver, were extremely ill, and had the same symptoms as those which were poisoned at Mallicollo. A little pig, the only one which we obtained at Tanna, having eaten the entrails of the fish, died soon after, being swelled to an unusual size."

From Wednesday night until Saturday night the Forsters were giddy and generally ill so that their investigations on New Caledonia were seriously impeded. On Saturday Forster writes:

"We still felt returns of dizziness which made us unfit for any kind of researches, in spite of ourselves, and even deprived us of the power of thinking, judging, and remembering, as well as of the perfect use of our external senses . . . The reader, who perhaps may find less food for his curiosity in this part of our narrative, than he expected, is requested to consider our corporeal and intellectual faculties were impaired by this virulent poison."

The following Tuesday the "Resolution" left New Caledonia, at which point Forster sums up the visit and describes the subsequent effect of the tetrodon poisoning:

"On leaving this place, we were far from being recovered, but had daily acute head-aches, and spasmodic pains over the body, together with an eruption on our lips. We also felt our selves much weakened, and unfit to go through our usual occupations."

From the foregoing accounts of this episode it seems reasonably certain that a virulent and concentrated poison, which manifests itself by disturbing the nervous system, resides in the liver, ovaries, or both, in the species in question. The fish was definitely of the genus *Tetrodon* Linn., and John Forster in his "Observations" on the same voyage indicated that it was related to *Tetrodon ocellatus*.

The first important investigation carried out in regard to the tetrodon poison was done by Geerts on the request of the Japanese government. His work was published together with other observations on fugu poisoning by Remy in 1883, and has served as a good foundation for most subsequent studies on the tetrodon poison.

Remy lists the common poisonous species as follows: *Tetrodon pardalis, T. rubripes, T. lineatus, T. vermicularis,* and *T. rivulatus.* Then, following the experimental feeding of cats and dogs and after inspection of case histories, he reaches two salient conclusions:

> "In the toxic fugus, the poison lies exclusively in the genital organs, principally the ovaries, and the toxic influence of the fish is proportional to the development of its genital organs.
>
> "The poison of the genital organs of the Tetrodons is a poison paralysing the nervous centers which has a more or less strong effect on the general and special sensibilities, and on the motility, and effects death by paralysis of the heart and suffocation."

The case histories upon which Remy was able to draw were published in France and have been unavailable to this author.

That tetrodon poisoning was not uncommon at the Cape of Good Hope is evident from Pappe (1866) who describes the situation in his *Synopsis of the Edible Fishes at the Cape of Good Hope:*

> "The highly poisonous nature of this small fish [*Tetrodon Honkenyi*] has been long known to the fishermen of the Colony, yet several persons have fallen a sacrifice from using it as food . . . in August 1845, two seamen belonging either to the Banka or Postillion, met with an untimely death, from having partaken of this fish, while the same fatal result, in August 1846, occurred to one of the crew of the French corvette *L'Oise,* who died on board of that vessel from the very same cause."

Since, at that time, no work had yet been done on the poison, Pappe makes some speculations as to the nature of the poison:

> "The exact nature of the poison has as yet been but little ascertained. It has been ascribed by some to the feeding of the fish on poisonous mollusca, by some to the disengagement of sulphuretted hydrogen, and by others again to a particular specific venom not yet discovered by chemical analysis. Whether the fish possesses that poisonous quality at all seasons, is not sufficiently known but it seems that most species belonging to the tribe are equally noxious. . . ."

Parts of the Pacific to which tetrodon poisoning is common besides China and Japan are Indo-China, Malaya, the Dutch East Indies,

and the Philippines. The common poisonous Philippine species, according to Seale (1912), is *Spheroides sceleratus*, locally called "tinga-tinga" or "botete." In addition, however, Seale states that there are thirteen other poisonous species of Tetraodontidae found in Philippine waters, and he says that, "Nearly all the natives of the Islands know that these fishes are poisonous, but either because of the peculiar flavor, or because of the ease with which they are caught, the fish are often eaten, and usually with deplorable results."

The problem of tetrodon poisoning in Malaya and Indo-China is, to say the least, twofold. Not only do individuals have to contend with poisoning from eating the fish but also from poisoning as a result of being bitten by it! Buddle mentions this fact in 1930:

> " . . . another fact concerning this thoroughly unpleasant creature—that is, its habit of attacking bathers or fishermen wading in the sea, and inflicting a nasty bite which becomes septic. Moreover, its favourite point of attack is upon the genital organs."

Gimlette, in his book on Malay poisons says the responsible species are *Tetrodon fluviatilis* and *T. oblongus*, common to Malay rivers. It is questionable whether the wound becomes septic from mucus in the fish's mouth, venom, or the nature and position of the bite. The situation in Indo-China is described by Salanoue-Ipin in his medical thesis on the "vulnérant" *Tetrodon fluviatilis*.

Returning for the moment to Buddle, it is interesting that this author also considers the roe and gall bladder to be the poisonous organs of the fish, and says:

> "The cleaning has to be done very carefully. It [*Tetrodon stellatus*] has to be skinned first, as the skin cannot be eaten and must not be cooked with the fish. The liver and gall-bladder have to be got out without breaking the gall-bladder. If this is broken, then the whole fish cannot be eaten."

Very little light has been shed on tetrodon poisoning in the West Indies. Disease from eating any fish is called "Ciguatera," whether it be produced from the inherent poison of the fish or from those toxins arising from putrefaction. Hoffmann, who has investigated the field of Ciguatera as a whole (1929), describes the general symptoms of Ciguatera, and states that fish of the genus *Tetrodon* are poisonous: "We have been shown that the tetrodon flesh is poisonous which has been used in California as small cakes spread out to kill stray dogs." Pellegrin, in his book on poisonous fish, notes that the

liver of *Tetrodon heraldi*, caught by local fishermen, was the organ used to make these lethal cakes for stray dogs in the La Paz section of Lower California. *T. heraldi* is known locally as "botete," the same name which is given to *Spheroides sceleratus*, poisonous in the Philippines. It should also be mentioned that Pellegrin gives a fine review of the poisonous species of the Tetraodontidae in various parts of the world.

The species responsible for what little poisoning occurs in Cuba are *Tetrodon testudinis* and *T. laevigatus*, commonly called "tambores." The ancient and widespread knowledge of the poisonous nature of tambores has reduced the problem to a minimum, which in part, at least, accounts for the fact that so little investigation has been carried out on the occurrence of the poison in the West Indies compared to that done by the Japanese. Thus, for example, little mention of tetrodon poisoning is made by Hill in Trinidad, again presumably because of the natives' taboo concerning the fish. It would appear, however, that the occurrence of Tetrodon poisoning in the West Indies would be an important step in determining the toxicity of other Atlantic species, particularly those of the east coast of the United States, and it is significant that tetrodon poisoning does occur in the West Indies.

TOXICITY AND CHEMISTRY

The Japanese, confronted with the problem of a delicious food fish being so poisonous, have taken up where Geerts and Remy left off. Their investigations from 1890 to the present day have been in three directions:

1. Determination of the localization of the poison in the tetrodon.
2. Isolation, purification, and determination of the chemical nature of the poison.
3. The poison's effect on its victims; symptoms and treatment.

The experiments on the localization of the tetrodon poison were begun by Miura and Takesaki in 1890 and were continued by Takahashi and Inoko in 1892. The former paper deals with one species, *Tetrodon rubripes*, each of whose organs these workers examined for presence of the poison between the end of March and the end of June. They extracted the poison separately from the ovary, liver, testes, kidneys, heart, spleen, skin, and skeletal muscle by macerating the tissues and using alcohol for extraction of the impure poison. It

should be mentioned, however, that Tahara (1910) showed that extraction with alcohol might be incomplete. Miura and Takesaki then injected the resultant extraction into rabbits and noted the effect and the time for reaction. They found that the most poisonous organ in *T. rubripes* is the ovary, which, however, is not poisonous when it is "spent." They noted that the effects of the poison on rabbits are cessation of respiration with the paralysis of the skeletal muscle, mydriasis, and cessation of heart beat.

The work of Takahashi and Inoko determined which of the species of *Tetrodon* common to Japan were the most poisonous and also what part of the fish was poisonous. They gave water extracts of the pulped organs to frogs and rabbits, and their results can best be presented in table form reproduced from their paper:

	Ovary	*Testes*	*Liver*	*The rest of the viscera*	*Muscle*	*Blood*
T. chrysops	+	+	+	+	−	n.e.
T. pardalis	+	+	+	+	−	+
T. vermicularis	+	+	+	+	−	+
T. poecilonotus	+	+	+	+	−	n.e.
T. rubripes	+	−	+	−	−	n.e.
T. porphyreus	+	−	−	−	−	n.e.
T. stictonotus	+	−	−	−	−	n.e.
T. rivulatus	+	n.e.	n.e.	n.e.	n.e.	n.e.
T. cutaneus	−	n.e.	−	−	−	n.e.

\+ Trace or more of poison present.
− No trace of poison.
n.e. Organ or tissue not examined.

Takahashi and Inoko also observed that the poison found in the blood of the two species examined was only a trace, and that in the ovaries the toxic substance was strongest during the spawning period in some species. Furthermore, these experiments show that in the tetrodons under examination, the ovary and the liver were the two most toxic organs and that the skeletal muscle was free from poison. The presence of poison in the blood, however, might make the body toxic if the fish remained uncleaned, although this seems improbable, since the poison which is in the blood apparently exists in such small amounts.

The purification and chemical study of tetrodon poison was begun in 1894 by Tahara. At that time he succeeded in isolating from the

ovaries of the ripe female fish an impure highly toxic substance which he called "tetrodon acid" because it gave a salt-like metal compound. In 1910, however, he improved the extraction and purification of this tetrodon acid and renamed it "tetrodotoxin," since it proved not to be an acid.

The method used in 1894 consisted, in its simplest form, of pulping the ovaries with water, separating the crystalline substances from the mixture by dialysis, and precipitating the raw impure poison with alcohol from the dialysate. The new method described in 1910, using over 1400 ovaries of *Tetrodon porphyreus* and *T. vermicularis*, was a great improvement over that mentioned above, since it obviated the necessity of the tedious dialysis. Most of the protein impurities were removed from the aqueous extract by acidification with acetic acid. The acid solution was treated with lead acetate and ammonia which caused the poison to precipitate along with lead hydroxide. After removing the lead with hydrogen sulphide, the filtrate was concentrated by evaporation. Part of the poison was precipitated with alcohol and the greater portion remained in solution.

The results of Tahara's work are as follows: Tetrodotoxin exists as a white, hygroscopic powder, very readily soluble in water and insoluble in the ordinary organic solvents. It consists of carbon, hydrogen, oxygen, and nitrogen; the provisional chemical formula is assigned as $C_{16}H_{31}NO_{16}$. The substance does not give any of the protein reactions nor is it precipitated by the alkaloidal reagents. The possibility that the poison is a protamine derivative is entirely excluded. The lethal dose with tetrodotoxin obtained by the new method is 4 mg. per kgm. body weight. In addition to the tetrodotoxin, a crystalline base, "tetronin," and a nitrogen free crystalline substance, "tetrodopentose," in smaller yield are always obtained. It is suggested that possibly the aforementioned substances do not exist in the organism as such, but are newly arisen through decomposition of the other products. On treatment with dilute hydrochloric acid the poison yields a crystalline base and an amorphous white powder of unknown character.

Other than statements to the effect that tetrodotoxin is neither a protein, an alkaloid, nor a protamine, Tahara does not go into the chemical structure of the poison. Subsequent investigations of tetrodotoxin have been directed at further purification and structural analysis of the poison.

Tahara's method of purification was used to obtain material used by Ishihara in 1924, at which time he stated that the poison is chemically an ester of glucose. This statement was challenged by Nagai and Ito (1939) because they found that "a reducing carbohydrate, which might have been considered the poisonous substance by Ishihara, is removed by means of $AgNO_3$ and seems to have no toxic value" and that "the nitrogen content in the purified poison is considerably higher than Tahara's." Some tests on the poison were done by Nagai and Ito and the following was deduced: all of the nitrogen may exist as amine, being attacked by HNO_2; 30% of the total amines are combined in amide form; the watery solution of the purified poison turns the polarization plane to the left; its $[\alpha]_D^{15}$ is $-$ 22.85; it seems probable that the poison is an acyclic compound. The authors did not use tetrodotoxin as purified by Tahara; instead they devised a different method and confined their efforts to *Spheroides rubripes.* They also intended to isolate the poison in crystalline form but were unsuccessful.

Apart from the Japanese work, recent studies by Macht and Spencer (1941) are of particular interest in that they deal with *Spheroides maculatus*, the Atlantic puffer. These investigators tested the toxicity of fresh muscle extracts of 65 different species of fish, both phytopharmacologically (by studying the growth of *Lupinus albus* seedlings) and zoopharmacologically (by intraperitoneal injections into white mice). They found that the extracts from nine species of fish, among which was *S. maculatus*, were "definitely toxic for growth of *Lupinus albus* seedlings," and that they were also "profoundly toxic for white mice." This was not true of extracts from the other species. The original experiments were made with fresh unheated suspensions, but Macht and Spencer showed that although heating destroyed the toxicity of most of the muscle extracts, those from the Tetraodontidae and Diodontidae could be heated to 60° C. without decreasing their toxicity to any great extent.

ACTION AND SYMPTOMS

It has been previously noted that the early investigator, Remy, described the action of tetrodotoxin on the body and made the following generality which still holds true:

> "The poison of the genital organs of the Tetrodons is a poison paralyzing the nervous centers which has a more or less strong

effect on the general and special sensibilities and on the motility, and effects death by suffocation."

Cases of the poisoning have been described by Cook, Forster, Remy, Takahashi and Inoko, and more recently (1927) by Leber. This latter case involves an Amboynese about thirty years old who became ill from eating *Tetrodon immaculata*. The symptoms are well described, especially the widespread and severe changes that occurred in the skin. The victim of the poisoning was treated successfully with intravenous injections of caffein, calcium, and stypticin (cotarnine hydrochloride). The symptoms and treatment show that in this instance the poison had great effect on the sensorium and vascular system.

The exact action of the poison, however, obviously must be determined by experimentation on animals as well as by the observation of specific cases in humans. The first group of these experiments was done by Remy and resulted in the statement quoted above. Ishihara (1924), in the same work wherein he describes experiments to determine the chemical nature of Tahara's tetrodotoxin, also describes work done to determine the pharmacological effect of the poison. He determined minimal lethal doses for about twenty different kinds of animals, both warm and cold blooded. Also he found that the paralysis affected the striated muscles and the autonomic nervous system, but did not affect the smooth muscles or the neuro-myo junction. This, however, was not confirmed by Kimura (see below).

The best work concerning the exact physiological disturbances of tetrodotoxin was done by Kimura in 1927. It is an extremely thorough study of the effects of Tahara's tetrodotoxin on the various organs and tissues of rabbits and frogs, both *in situ* and excised. He found a number of important facts, which can be summarized as follows: Tetrodotoxin loses its poisonous properties relatively quickly in animal bodies through some as yet unknown mechanism. But the poison is not detoxified due to the action of kidneys, liver, or muscle tissue. Some poison may be lost due to the alkalinity of the blood, but not enough to account for such a great difference in toxicity when the poison is injected slowly rather than quickly. Only adrenalin hinders the resorption of the tetrodotoxin through vasoconstriction, thus allowing the animal to bear more than the minimal lethal dose. Adrenalin, consequently, may be used effectively in the treatment of tetrodon poisoning under certain circumstances. The poison affects

the intestine by lowering tonus; with larger doses it increases tonus. These effects may be due to a paralysis of the vagus first, and then, with increasing amounts of the poison, paralysis of the sympathetic division. Tetrodotoxin affects the isolated living heart of the rabbit and the frog by decreasing the amplitude and frequency of the beat to a final diastolic stand-still. The poison has three successive actions on the heart—namely, paralysis of the impulse pathway so that sino-auricular and auriculo-ventricular dissociation is brought about, paralysis of the impulse center during which period the heart muscle will respond to mechanical stimulation, and finally, paralysis of the contractile fibers.

Yano, in 1938, experimenting with frogs and rabbits, attempted to determine whether the two most serious effects of tetrodon poisoning, *i. e.*, difficulty in breathing and a sharp decline in blood pressure, were due to inhibition (paralysis) or excitation, central or peripheral. Again tetrodotoxin prepared according to Tahara's method was used. Yano reached the following conclusions: the vasomotor center, course of the cardiac impulse, and cardiac muscle are paralyzed; tetrodotoxin paralyzes not only the vasoconstrictor nerves, but also the muscle of the blood vessel itself; in that case the decrease of blood pressure is caused by paralysis of the vasomotor center, vasoconstrictor nerves, peripheral blood vessels, and cardiac muscle; the poisonous action is very transient.

Concerning the treatment of cases of tetrodon poisoning, Yano goes on to say:

> "One of the remarkable characteristics of this toxin is that the poisonous action is very transient. This makes the symptomatic treatment very promising. The most excellent result was obtained by coramin (cornidin) on the isolated frog heart as well as on the respiration and the blood-pressure of a live-rabbit. Tyramin is effective on the descending of blood-pressure. Coffein, lobelin, natrium-thiosulfate are potent for the disturbance of respiration. The intravenous infusion of much physiological salt solution combined with the above medicines gives a still better result."

DISCUSSION

From what has been said it is immediately apparent that poisoning by the Tetraodontidae has been recorded for at least two and a half centuries, and it is not unreasonable to suppose that it has been known for a much longer time. There has been relatively little work, however, on the possible toxicity of the Atlantic puffer, *Spheroides maculatus* (Bloch and Schneider), which has recently attained market status. This is explicable because the fish has been eaten to such a limited extent; also, if it be toxic and has caused any illness at all, the cases have been so rare as to attract little attention.

The review of the literature presented here indicates that in many Tetraodontidae the poison is associated with the gonads and liver, and there is also evidence that the degree of toxicity varies in some species and may be most severe at or near the time of spawning. If *Spheroides maculatus* is at all toxic, there are two factors, apart from its limited consumption, which have in all likelihood played a part in the failure to detect this condition. First, it has probably been efficiently cleaned and eaten shortly after its capture at points near the fisheries which take this species. Second, its consumption by humans has been mainly confined to the summer and early fall months, when for example it is taken most abundantly by Long Island fishermen; this period is, in the main, *after* the spawning season (Warfel, Merriman, and Olsen—MS.).

In view of the information presented herein, it is evident that toxicity tests should be undertaken on a seasonal basis and under varying conditions wherever this species is offered for sale. It is hoped that this paper may be of value in stimulating such research and in providing a review of the available pertinent information. Until such research is completed it would seem expedient if special care were exercised in the preparation of the common puffer, *Spheroides maculatus*, for market.—D. M.

REFERENCES

*Bennett, G.
1870. On the "toadfish" (*Tetrodon hamiltoni*) of New South Wales. N. S. W. Med. Gaz. **1**: 176 ff.

Blyth, A. W.
1920. Poisons: Their effects and detection. 5th ed., 496–497. London.

*Boesoirie, C.
1940. Fish poisoning due to "ikan nogi-nogi." Geneesk. Tijdschr. v. Nederl.-Indie. **80**: 1338–1340.

Buddle, R.
1930. Some common poisonous fishes found in Singapore water. Journ. Roy. Navy Med. Serv. **16**: 102–111.

Cook, J.
1777. A voyage towards the south pole and round the world. **2**: 112–113. London.

Forster, G.
1777. A voyage round the world. **2**: 403–411, 423–424. London.

Forster, J. R.
1778. Observations made during a voyage round the world. pp. 210–211. London.

Gimlette, J. D.
1915. Malay poisons and charm cures. pp. 21–23. London.

Gudger, E. W.
1930. Poisonous fishes and fish poisonings with special reference to Ciguatera in West Indies. Am. Journ. Trop. Med. **10**: 43–55.

Herre, A. W. C. T.
1940. Fisheries and fishes. Andhra Univ. Coll. Mag. and Chron. **3** (6): 12.

Hill, R.
1868. On poisonous fishes. Proc. Sci. Ass. Trinidad. **1**: 221–222.

Hoffmann, W. H.
1927. La Ciguatera, die Fischverigiftung von Cuba. Arb. u. Tropenkrankh. (Festschr. B. Nocht) pp. 197–200.
1929. La Ciguatera, enfermedad producida por peces venenosos de Cuba. Investigation Y Progresso. **3** (11): 101.

Ishihara, F.
1924. Studien über das Fugutoxin. Archiv. für Exp. Path. u. Pharmak. **103**: 209–218.

Jordan, D. S.
1925. Fishes. pp. 622–636. New York.

Kaempfer, E.
1728. The history of Japan (Translated by J. G. Scheuchzer). **1**: 134–135.

*No attempt has been made in this review to include literature printed in the Japanese language. A few of the references included in the bibliography are not mentioned in the body of the paper, and those items preceded by an asterisk have not been examined in the original by this author.

KIMURA, S.
1927. Zur Kenntnis der Wirkung des Tetrodongiftes. Tohoku Journ. Exp. Med. **9**: 41–65.
LEBER, A.
1927. Über Tetrodonvergiftung. Arb. u. Tropenkrankh. (Festschr. B. Nocht) pp. 641–643.
MACHT, D. I. AND E. C. SPENCER
1941. Physiological and toxicological effects of some fish muscle extracts. Proc. Soc. Exp. Biol. and Med. **46**: 228–233.
MIURA, Y. AND K. TAKESAKI
1890. Zur Localisation des Tetrodon-Giftes. Arch. Path. Anat. **122**: 92–99.
NAGAI, J. AND T. ITO
1939. Chemical study of fugu (Spheroides) poison; nitrogen distribution in purified poison. Jour. Biochem. **30**: 235–238.
NIGRELLI, R. F.
1935. The morphology, cytology and life-history of *Oodinium ocellatum* Brown, a dinoflagellate parasite on marine fishes. Zoologica. **21** (3): 129–164.
OSBECK, P.
1771. A voyage to China and the East Indies. (Translated by J. R. Forster.) **1**: 364–366. London.
PAPPE, L.
1866. Synopsis of the edible fishes at the Cape of Good Hope. 2nd edition, pp. 4–5. Capetown.
PELLEGRIN, J.
1900. Les Poissons Veneneux. pp. 37–50. Paris.
REMY, C.
1883. Sur les poissons toxiques du Japon. C. R. Mem. Soc. Biol. **35**: 1–28 (Mem.).
SALANQUE-IPIN, H.
1889. Etude Sur un Tetrodon Vulnérant du Cambodge. 42 p. Bordeaux.
SEALE, A.
1912. Some poisonous Philippine fishes. Phil. Journ. Sci. sec. D. **7**: 289–293.
*TAHARA, Y.
1894. ———— Zeitschr. d. japan. pharmaz. Gesellsch. vol. 14. (Cited in Tahara, 1910.)
1910. Über das Tetrodon-Gift. Biochem. Zeitschr. **30**: 255–275.
TAKAHASHI, D. AND Y. INOKO
1892. Beitrage zur Kenntniss des Fugugiftes vom Fugufisch. Mitth. Medic. Facult. Tokio. **1** (5): 375–402.
WHITLEY, G. P.
1943. Poisonous and harmful fishes. Austral. Coun. for Sci. and Indust. Res., Bull. 159.
YANO, I.
1938. An experimental study on the globe-fish (Fugu) intoxication. Jap. Journ. Med. Sci. (VIII Int. Med. Pediat. and Psychiat.). **5**: 99–101.