MARINE BIOTOXINS: ISOLATION AND PROPERTIES[1]

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I. Introduction

Marine biotoxicology is concerned with poisons produced by marine plants and animals. Since the literature dealing with marine algae has been adequately reviewed by Schwimmer and Schwimmer (93), this review is limited to toxic agents produced by animals. Biotoxins are widely distributed within marine animals, viz., protozoa, sponges, coelenterates, molluscs, annelids, arthropods, echinoderms, fish-like vertebrates, reptiles, and mammals. The literature dealing with the chemistry of the toxins of these organisms will be presented in a phylogenetic sequence beginning with the protozoa. The subtopics concern isolation studies and a summary of available information on chemical composition. It is not within the scope of this review to refer to the more than one thousand articles which have appeared on such facets of marine biotoxicology as morphology, epidemiology, zoögeography, ecology, clinical characteristics, and pharmacology.

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II. Sources of Marine Biotoxins

A. SHELLFISH (via PROTOZOA)

Marine protozoans poisonous to man appear to be limited to a single order of mastigophorans, the *Dinoflagellata*. They abound in neritic waters and in the high seas, ranging from tropic to polar oceans. Dinoflagellates form an exceedingly important part of the ocean plankton as producers of carbohydrates, proteins, and lipides. During their periodic maxima they cause local areas of discoloration of the sea which have been designated as red water, red current, or red tide. The "blooming" of these toxic plankton in excessive numbers frequently causes a mass mortality of the fishes living in the region involved. However, this discussion will be limited to the toxic dinoflagellates which are known to be involved in human poisonings. On the basis of many field and laboratory observations (57, 73, 98), it has been conclusively demonstrated that certain species of toxic dinoflagellates, viz., *Gonyaulax catenella*, *G. tamerensis*, *Pyrodinium phoneus*, and possibly other species, are the primary cause of paralytic shellfish poisoning. The symptoms of paralytic shellfish poisoning result from ingesting bivalves which have become toxic owing to their feeding on poisonous dinoflagellates.

The problem of the chemistry of mussel toxin has attracted unusual interest in the Western United States because the mussels in certain areas along the Pacific Coast seasonally become toxic and are subjected to quarantine. The study of the chemistry of the toxic principle in mussels was introduced by Salkowski (91) as a result of a mass poisoning that occurred in Wilhelmshafen, Germany, from eating mussels. Salkowski carried out only elementary fractionations based on solubility properties. Brieger (13, 14, 15) undertook the isolation of the toxin and obtained a fraction which formed an insoluble compound with gold chloride. From the chemical properties and analytical data, it was concluded that the toxin was trimethylglycerylammonium hydroxide (15), However, large discrepancies were found to exist between the natural material and the synthetic compound.

Richet (87, 88) described the isolation of a toxic fraction from *Mytilus edulis*, but the physiological properties left considerable question as to whether the toxin was the same as that reported by Brieger. Because of the similarity of physiological action of the toxin from *M. edulis* to that of congestin from actinias (82, 83, 84), Richet named this mussel toxin mytilocongestin. The problem of the chemical nature of mussel toxin then remained dormant for nearly two decades until Ackermann (2) attempted a fractionation of toxic mussel tissue. A number of compounds were isolated and identified, but no evidence was found to indicate the presence of the toxin described by Brieger. Meyer, Sommer, and Schoenholz (64) attempted a purification of the toxin but gave no details as to their method other than that solubility properties were utilized.

A critical study of the problem of isolation of mussel toxin may be considered as beginning with the work of Müller (70). Thereafter, the major attention was given to the toxin that produces paralytic symptoms and is commonly referred

to as paralytic mussel poison. Since the same or a similar toxin has been observed in shellfish other than the mussel, the term paralytic shellfish poison has also come into common use. The toxin would seem to be the one whose isolation as the chloroaurate was claimed by Brieger.

Müller (70) examined critically each step in the isolation procedure of Brieger and found that the toxin is not precipitated by gold chloride and that it undergoes rather complete destruction when left in the presence of gold chloride solution for any extended period. It was Müller's conclusion that Brieger's toxic fraction consisted of inert material contaminated with small amounts of residual toxin.

Acidified methanol proved to be an excellent extracting solvent, but attempts to find a satisfactory precipitating agent were not successful. Rufianic acid and Reinecke acid were found best, but these gave erratic results and incomplete precipitation accompanied by considerable destruction of the toxin. Of the numerous adsorbing agents tried, permutit was found most ideal and gave the first clue that the toxin was a strong organic base. By combining the best methods of extraction, ion exchange, and precipitation as the Reineckate followed by conversion to the hydrochloride, Müller obtained a fraction with a toxicity of 3.6 gammas per "mouse unit," the mouse unit being defined as that amount of material which, upon intraperitoneal injection, produces death in a 20-g. mouse in 10–20 min. (80, 94). (Unless otherwise stated, this unit should be understood when subsequent toxicity values are given.) The principal impurities remaining with the toxin were found to be inorganic, and attempts to remove this inorganic material always resulted in some destruction of the toxin.

Improvements in the ion-exchange procedure followed the work of Müller. Monnier (67) found that barium permutit was more efficient than the sodium form as an adsorbant, and Bendien and Sommer (12) learned that the toxin could be adsorbed from acidified aqueous medium by Norit A if the concentration of the toxin was not too small. Using Norit A, the toxin was obtained with lesser amounts of inorganic contaminants than Müller's product and with the same order of toxicity. Other adsorbing agents have been investigated (97), but none was found as efficient as those already in use.

A large amount of quantitative data of a critical nature has been made available by the reports of Mold (65) and Wikholm (111). These workers examined quantitatively the various steps in the isolation procedure and introduced a number of refinements with the aim of making possible the isolation of the toxin in quantities sufficient to allow more extensive study of its chemical properties and structure. A new precipitating agent was found in helianthic acid (65). Although not ideal, it was usable as a starting point for conversion to the hydrochloride as an analytic form. By means of these refined methods, Mold purified the toxin hydrochloride to a toxicity value of 0.25 gamma per mouse unit. Details of further improvements in the isolation procedure have recently appeared (66). Use is made of Amberlite IRC-50 ion-exchange resin followed by chromatography on the hydrogen form of Amberlite XE-64 and on acid-washed alumina. The hydrochloride of the toxin was obtained in low yields as an ash-free amor-

phous product which had a toxicity of less than 0.2 gamma per mouse unit. Evidence is presented (66) for the high degree of purity of this product based on data from countercurrent distribution of the dihydro derivative of the poison.

Mussel toxin is dialyzable (64). Its specific rotation $[\alpha]_D^{25} = +103° \pm 5°$ (66). It is soluble in water, methanol, hot ethanol, aqueous acetone, and glacial acetic acid. The solubility in nonacidic solvents is enhanced by the presence of a small amount of acid (66). It is insoluble in ether, chloroform, ethyl acetate, butanol, and the hydrocarbon solvents (96). A slow deterioration of the toxin occurs on dry storage of mussel tissue (70), but this can be inhibited with alcohol or chloroform. Rapid loss of toxicity occurs on boiling in acid or treatment with alkali in the cold, but destruction is not marked on boiling in neutral solution for brief periods. The toxin is unstable in the presence of heavy metal salts (70). Wikholm (111) tried without success to convert the hydrochloride to the free base by means of silver hydroxide.

The toxin does not give aldehyde reactions or a diazo reaction and is not reduced by Fehling's solution. Bromine water and permanganate do not react in the cold, indicating the absence of double bonds. Addition of alkali results in a fish-like odor readily distinguishable from that of trimethylamine (70). Infrared spectrophotometric analysis (65) gives no evidence of double bonds but does give unquestionable evidence for the presence of CH and either NH or OH groups. The C=N group is indicated and it is assumed that this is part of a guanidine group, the presence of which had been demonstrated by the Sakaguchi test. Carboxyl groups are absent, as indicated by a negative hydroxamic acid test with ferric chloride. The best analytical form is the hydrochloride, which is formed as a glassy, amorphous, hygroscopic mass on evaporation of the hydrochloric acid solution of the toxin. Elemental analysis of the hydrochloride suggests the empirical formula $C_{10}H_{17}N_7O_4 \cdot 2HCl$, and this formula is in acceptable agreement with the molecular-weight value obtained by the cryoscopic method (66).

A report from oriental workers (46) challenges the validity of much of the work of American investigators. These workers point out that venerupin, a toxin long known in Japan, is changed on treatment with acidified methanol to yield a second toxin whose physiological properties are similar to those produced by paralytic shellfish poison. This artifact is believed to be an ester derivative of betaine. It is not possible at this time to evaluate the significance of this observation. The presence of betaine as an impurity in the later stages of purification of mussel toxin has been reported (96), suggesting that the toxin has some properties in common with this substance.

Although the recently discovered dinoflagellate *Gymnodinium veneficum* Ballantine (11) has not, according to present knowledge, been responsible for the poisoning of humans, mention is made of this dinoflagellate here because of the potent toxin that it contains (1). The poison is soluble in water and the lower alcohols but insoluble in ether and chloroform. It is very sensitive to hot alkali and dilute acid, the products of its acid-catalyzed decomposition also being toxic. Because of its inability to pass a membrane in dialysis, the toxin molecule is assumed to be large and to have a molecular weight greater than 1000.

B. SPONGES

Poisonous sponges are among the least studied of toxic marine organisms. Toxic substances have been reported as present in sponges by Richet (86), DeLaubenfels (26, 27, 28, 29), and Halstead and Habekost (43). There are no data available regarding the chemistry of these substances.

C. COELENTERATES: HYDROIDS, JELLYFISHES, SEA ANEMONES, CORALS

A dominant characteristic of this group is the presence of tentacles equipped with complex stinging organs known as nematocysts. The nematocysts of certain species of jellyfishes produce potent chemical toxins which have been known to produce death in humans within a few minutes.

Four toxic principles have been reported as occurring in species of *Coelenterata*. Two of these, thalassin and congestin, have been found in the tentacles of the sea anemone, and distinction between these is made on the basis of the difference in solubility in alcohol (81, 85). Ackermann, Holtz, and Reinwein (3) established beyond question the identity of thalassin to tetramethylammonium hydroxide. Proof of identity was found in the coincidence of physical properties of a number of derivatives of the toxin with those of the same derivatives prepared from this quaternary base. The only question that remains is whether all of the physiological properties ascribed to thalassin can be accounted for in terms of tetramethylammonium hydroxide. A subsequent study by Sonderhoff (99) did not provide an answer to this question.

The toxin known as congestin is presumed identical to the congestin observed by Richet (82, 83, 84) in the actinias. Congestin may possibly be identical or related to mytilocongestin, which was observed by this same worker in mussels (87, 88) but was not further investigated. The congestin of *Coelenterata* is insoluble in 50 per cent alcohol but soluble in dilute bicarbonate solution. It has the nitrogen content of a protein but is heat stable at autoclave temperatures for brief periods (82).

Portier and Richet (79) reported a heat-sensitive, nondialyzable toxin in the filaments of the *Physalia*. This poison, called hypnotoxin, seemed to have protein properties. The heat sensitivity of the toxin was later corroborated and it was found that the toxin is digestible by trypsin (25). Nothing further is known of the chemistry of this toxin or that of medusocongestin obtained by Dujarric de la Riviere (31) from an extract of medusae.

D. MOLLUSCS

Molluscs are unsegmented invertebrates having a soft body and usually secreting a calcareous shell. The three classes considered to be of importance to the biotoxicologist are: *Gastropoda*, the univalves; *Pelecypoda*, the bivalves; and *Cephalopoda*, which include the octopus and squid.

1. Gastropoda

The most dangerous members of this group to man are members of the genus *Conus*, the venomous cone shells. Unfortunately, there are no data available regarding the chemical nature of their venom.

A second group of interest are certain species of the genus *Murex*, from which is obtained the toxic substance murexine (33). The early work of Dubois (30) and of Roaf and Nierenstein (89) provided little of significance on the chemistry of the toxin. A possible structural relation of the toxin to acetylcholine has been suggested (50, 51, 52) based on the similarity of physiological action in muscular contraction to that of acetylcholine. Such a relation was demonstrated by Erspamer and Dordoni (34) by showing that the loss of the acetylcholine-like action on hydrolysis could be restored by acetylation of the products of hydrolysis. Proof of the structure of murexine as β-(imidazolyl-4)acrylcholine has been described (32), and its synthesis has been accomplished (76).

2. Pelecypoda

Most of the poisonings produced by molluscs probably result from paralytic shellfish poison. The causative agents are the dinoflagellates (see Section II,A). There is, however, a second type of shellfish poisoning, known as venerupin poisoning, in which the dinoflagellates do not appear to be involved. The disease is restricted to certain regions in Japan and follows the ingestion of Japanese *Tapes* and *Ostrea*. Studies on venerupin were prompted by a mass poisoning which occurred in the Lake Hamana area of Japan in 1942. The available chemical information on the toxic principle is limited to observations on solubilities in various solvents, stability, and precipitating agents (4, 5, 6). A method of isolation and purification which uses several alcoholic extractions, dialysis, and chromatography on alumina and paper has recently been described (49). The toxin differs from paralytic shellfish poison in that it is not precipitated nor destroyed by Reinecke acid and differs from tetrodotoxin in having a much greater stability to heat over a wide range of pH and a much lower degree of toxicity.

3. Cephalopoda

Cardio-inhibitory and vasopressor substances have been found to be present in the salivary glands of octopi. Similar substances are believed to be present in squid and other cephalopods. The salivary glands of the *Octopus vulgaris* are reported to contain a toxic principle. Livon and Briot (61) seem to differ with Henze (47) on such simple properties as solubility in alcohol and stability to heat, facts which indicate the meager amount of reliable information available with reference to the chemical nature of the toxin. Neither these workers nor de Rouville (90) proceeded further than obtaining simple extracts of the salivary glands. Baldwin (10) refers to agmatine as a toxic substance in the saliva of the octopus but gives no reference for his statement. More recently, Bacq and Ghiretti (8, 9) have shown that the venomous effects of the saliva from the posterior salivary glands of *Octopus vulgaris* are not due to tyramine, histamine, or to any combination of these. A paper chromatogram of the saliva indicated the presence of 5-hydroxytryptamine, tyramine, tyrosine, and a phenolic amine called octopamine.

E. ARTHROPODS: HORSESHOE CRABS

A few species of marine arthropods are known to be toxic. Vague references appear in the literature regarding the toxicity of certain species of tropical crabs. *Emerita analoga*, the common Pacific Coast sand crab, has been found to contain paralytic shellfish poison (95). *Carcinoscopius rotundicauda* and some species of *Tachypleus*, Asiatic horseshoe crabs, have been known to produce fatal poisonings in humans by ingestion of their eggs and flesh. There are no data available concerning the chemical nature of the poison.

F. ECHINODERMS: STARFISHES, SEA URCHINS, SEA CUCUMBERS

Although starfishes have been reported as poisonous (36, 42, 77) and some sea urchins are known to contain venom organs (38, 109), our meager knowledge of the chemistry of echinoderm poisons is limited to the toxin of the sea cucumber. This toxin, which seems to be concentrated in the "pink gland," was first reported by Nigrelli and named holothurin (74). The toxin, as extracted from the glands of *Actinopyga agassizi* with ethanol, is a neutral substance, apparently free of nitrogen, and shows no absorption in the ultraviolet region. The specific rotation is reported to be $[\alpha]_D^{25} = -19°$ (75). After purification on a mixed-bed ion-exchange column, holothurin shows an acid equivalent of 1148 and analyzes for $C_{50}H_{82}O_{26}S$. The sulfur exists as sulfate in an ester linkage (24). Acid hydrolysis of holothurin yields a mixture of insoluble aglycons and three water-soluble sugars (probably rhamnose, xylose, and glucose) in a ratio of 2:1:1. The aglycons are postulated to be closely related to one another and to consist of a steroidal skeleton with two conjugated double bonds and five or six oxygen functions (75). Purified holothurin, after hydrolysis with methanolic hydrochloric acid, yields an aglycon which has been called holothurinogen (24). An infrared spectrum indicates the presence of hydroxyl and carbonyl groups and double bonds. Holothurin is reported to have anticarcinogenic properties (74).

G. CHORDATES: FISHES

The literature on poisonous and venomous marine vertebrates is primarily concerned with fishes. A number of distinct poisons are believed to exist in a wide variety of fish-like vertebrate species, but the chemical identity and relationships have not as yet been defined. Consequently, clinical terminology now in use may require modification as additional chemical information becomes available. Ichthyotoxism is a general term which should be used to include the forms of poisoning resulting from contact with both poisonous and venomous fish-like vertebrates. Poisonings from venomous fish are termed ichthyoacanthotoxism; those from poisonous fish are referred to as ichthyosarcotoxism, ichthyoötoxism, or ichthyohemotoxism depending on the presence of the toxin in the flesh, roe, or blood of the animal, respectively.

1. Ichthyosarcotoxins

One of the more serious forms of ichthyosarcotoxism results from the ingestion of the flesh of certain species of moray eels and has been aptly termed gymno-

thorax poisoning, after the generic name of the fish. Most of the eel species in which this poison is found are inhabitants of the tropical Pacific. Aside from a minor note on the dialysis of gymnothorax poison by Halstead and Ralls (44), few chemical data are available.

The marine toxins discussed thus far have been found to occur without question in the living animal. Another type of toxin which seems to develop *post mortem* has been reported. This form of ichthyosarcotoxism is termed scombroid poisoning, since it usually results from eating scombroid mackerel-like fishes. The toxic agent has been regarded as related to histamine or histamine-like substances. A study on the relative amounts of histamine in fresh and stored fish (41) revealed that histamine is present only in small amounts in fresh California sardines, mackerel, and albacore, but that the concentration of histamine increases rapidly *post mortem*. Markov (63) attributed the formation of histamine to enzymes and bacterial flora. Studies on the incubation and hydrolysis of fresh and stored fish suggested that the histamine must exist in fresh fish as a peptamine from which the histamine is released by bacterial action after the death of the fish (41). As convincing as this conclusion seemed to be, the position was reversed in a later report (39), when it was found that bacteria are incapable of decarboxylating peptamine to yield histamine. A number of cases of fish poisoning have been reported from eating tuna which on examination showed the presence of so few bacteria that a bacterial origin of the toxin seemed highly questionable (60).

The conclusion that the toxic material is histamine has been challenged by Kawabata, Ishizaka, and Miura (54, 55). Paper chromatographic analysis showed that the R_f value of the toxin differed from that of histamine. Further doubt that histamine is the active agent comes from a recent article by Geiger (40). Reference is made to the work of Feldberg and Schilf (35), who point out that oral administration of large doses of histamine does not prove toxic to animals, because of destruction or detoxification in the intestinal tract. Geiger did find, however, that if the intestinal mucosa is first damaged by saponin, the oral administration of histamine produces the symptoms of histamine poisoning. The name "saurine" has been applied to this new vagus stimulant, which resembles histamine in its action (56).

Ciguatera is one of the most important forms of ichthyosarcotoxism from the viewpoint of its public health significance. It is estimated that more than 300 species of fish have been incriminated. Most of these are inhabitants of warm seas, particularly the Caribbean Sea and the Pacific and Indian Oceans. The only recently reported attempt to study the chemistry of ciguatera toxin (45) was stimulated by an outbreak of poisoning which occurred on May 20, 1949, in Tokyo. The remains of the single barracuda which had produced the mass poisoning were examined, and it was demonstrated that the contained toxin was different from puffer toxin and paralytic shellfish poison, since it was soluble in the fat solvents. The toxin was also found to be soluble in methanol, acetone, and water, but insoluble in water made acid with tartaric acid. Since the toxic symptoms were observed in people who ate the boiled fish, it is apparent that the toxin has a degree of stability to heat.

Puffer or tetraodon poisoning is the most violent form of ichthyosarcotoxism known. The disease is caused by eating any one of a large variety of puffer-like fishes. The extreme toxicity of Japanese puffers and their therapeutic potential were recognized in the Orient as early as the 16th century (100). Experimental research on the subject was first conducted by Masamori Fukushima of Japan in 1716. Captain Cook and several officers of His Majesty's ship The Resolution were almost forced to terminate their famous world voyage of 1774 because of poisonings which resulted from ingestion of a poisonous puffer. The clinical reports of these incidents by Anderson (7) and Forster (37) have become classics in marine biotoxicology. Captain Cook's episodes were, however, merely a prelude to scores of clinical accounts that made their appearance in the scientific literature of the 19th and 20th centuries.

Most of the toxic species of puffer are tropical but some are found in temperate zones. While extracts of puffer toxin were prepared by Takahashi and Inoko (103, 104, 105) and by Yashizawa (112), research on the chemistry of puffer toxin may be considered as initiated by Tahara (101). Much of this work is now of historical interest only, but of possible significance yet are his findings that an impurity, later identified as a mixture of scillitol and mesoinositol (106), remained with the toxin down to the last stages of his process of purification. This suggests that the toxin has some properties in common with the polyhydroxy compounds. It was Tahara also who made the first and only significant attempt to date to study the hydrolysis products of the toxin (102). The apparent reason for absence of later work in this area is evidently related to the unavailability of sufficient quantities of the purified toxin. Tahara isolated two hydrolysis products, one of which was an acid and the other a nitrogen-containing compound precipitated as the gold salt or as the picrate. Analytical data on these derivatives were consistent with the formula $C_4H_9O_2N$. If it were known with certainty that the hydrolysis product was from the toxin and not from some impurity, this information could be of inestimable value in establishing the structure of the toxin.

Ishihara (48) observed that the reducing properties of the toxin increased on hydrolysis and on this basis concluded that a reducing carbohydrate was an integral part of the toxin molecule. Isolation of glucose as the osazone from the hydrolysis mixture seemed to confirm this conclusion. However, such a conclusion is subject to the uncertainty which arises because of contaminated starting materials, and the probability remains that this glucose was a hydrolysis product of an impurity. The conclusion of Ishihara has been challenged by Nagai and Ito (72), who found that glucose could be obtained from the crude toxin by methods short of hydrolytic action. An examination of the reports by Ishihara and Nagai indicates that neither of these workers was employing other than a crude preparation of the toxin.

Nagai and Ito (72) concluded that all of the nitrogen of the toxin was amino nitrogen and that two-thirds of this was involved in an amide linkage. This conclusion is also subject to considerable question, since the only criterion of purity used was that the total nitrogen was essentially the same as amino nitro-

gen by analysis. Further evidence for the gross impurity of the samples used is seen in the large discrepancy which exists between the reported specific rotation value ($[\alpha]_D^{15} = -22.85°$) as compared to the more recent data of Tsuda and Kawamura, who give the specific rotation as $[\alpha]^? = -8.64°$ (108).

The problem of the structure of puffer toxin was further confused by the report of Kanayama (53) concerning the isolation of a toxic fraction which contained phosphorus but no nitrogen. It seems that no subsequent worker has given more than a passing notice to this report. Kanayama used a different method of isolation and it is impossible at this time to tell whether he had isolated a fraction which contained Tahara's toxin as a minor contaminant or whether he had isolated a different toxin.

Puffer toxin was isolated by Yokoo (113, 114) in a form believed to be quite pure, and the formula $C_4H_7O_3N$ was suggested on the basis of analytical data and a molecular-weight value of 116. In a later report (116), this molecular-weight value was revised to 335 and a formula essentially three times the previous one was suggested ($C_{12}H_{17}O_{10}N_3$). Equivalent-weight determinations supposedly based on the titration of an amine group and a lactone group gave values in the area 350–365 (116). The concept of the presence of a lactone ring was subsequently abandoned, and the titration values were assumed to be derived from the neutralization of the amine hydrochloride (117). An improved procedure for isolation has been described by Yokoo (115), but this method did not come into general use because of the development of more effective chromatographic methods (108). Using these chromatographic methods in conjunction with recrystallization, Tsuda and Kawamura (108) obtained a product with a constant toxicity of 0.01 gamma per gram of mouse weight. Although the purified toxin was subjected to elemental analysis and equivalent-weight determinations, no empirical formula was suggested. The analytical data are in good agreement with $C_{11}H_{19}O_8N_3$, a formula which has a lower molecular weight than the values uniformly obtained experimentally. Infrared spectrophotometric analysis indicated the presence of OH (or NH) and $CONH_2$ (or C=N) groups. Ester, lactone, lactam, aldehyde, and ketone carbonyl groups are absent.

Evidence is presented from a recent study by Yokoo and Morosawa (117) for the existence of two forms of the toxin which differ in toxicity but which give identical infrared spectrograms and paper chromatograms. The spectrograms are essentially identical to that obtained by Tsuda and Kawamura (108), and the analytical data for one form are also in good agreement with the data obtained by these workers.

A process for the isolation and purification of puffer toxin has been described (71) which utilizes Amberlite IRC-50 and IR-4B ion-exchange resins. The purified product had a toxicity of 0.008 gamma per gram of mouse weight, a value comparable to that of the best products obtained by Tsuda (108) and Yokoo (117). Repeated recrystallizations, however, failed to result in constant analytical values for carbon, hydrogen, and nitrogen.

Much of the data on the physical and chemical properties of puffer toxin are conflicting, owing to the differences in composition of the toxin samples used by

various investigators. The following properties seem to be established with a reasonable degree of certainty. The toxin when pure is slightly soluble in water (107) but becomes readily soluble in impure mixtures (102). It crystallizes as silky or prismatic needles which have no definite melting point but which begin to carbonize at about 240°C. (71). The poison is not precipitated by any of the protein or alkaloidal precipitating agents but is precipitated by ammoniacal lead acetate (102), copper hydroxide, iron hydroxide, silver hydroxide, and rufianic acid (114). It is unstable in alkali but relatively stable in acid, and is destroyed by prolonged boiling in water (102). The toxin does not give a ninhydrin test for α-amino acid (108) nor does it give positive tests for the purine ring or the guanidine group (72). As yet no investigator has ventured a supposition as to the structure of the toxin.

2. Ichthyoötoxins

With few exceptions ichthyoötoxic fishes are freshwater species; therefore, they do not come within the scope of this presentation.

3. Ichthyohemotoxins

The name ichthyotoxicum was applied by A. Mosso (68) to the toxic principle in the serum of the anguilla, moray, and conger eels. It has been assumed from earliest times that the toxins from the various eels are identical, though most of the investigations have been made on the moray eel. The toxin of the serum does not survive passage through the digestive tracts of animals and symptoms are apparent only upon injection. Two types of physiological properties have been observed, one being hemolytic in nature and the other toxic. The early reports of Camus and Gley (20, 21, 22, 23), Kopaczewski (58, 59), Lumiere (62), and Buglia (17) deal largely with an extended controversy as to whether the hemolytic properties are due to the same factor that causes the toxic symptoms. The hemolytic factor seems to be related to a salt of a higher fatty acid (17).

The early reports by A. Mosso (68), U. Mosso (69), Camus and Gley (19), Kopaczewski (59), and Phisalix (78) on the heat-labile nature of the toxin now appear to be in error. The apparent loss of toxicity on heating was evidently to be explained on the basis of adsorption of the toxin on protein materials which were coagulated by the heat. Buglia (16) demonstrated that the toxic properties may be partially recovered from the coagulum by enzymic digestion of the protein or by mechanical separation such as is produced by grinding with sand. The toxin is reversibly unstable to acids and irreversibly unstable to treatment with alkali (69). The question whether digestive destruction is due to the action of gastric hydrochloric acid or to enzymes seems not to have been satisfactorily answered. U. Mosso (69) concluded that the stomach acid was sufficient to account for the destruction without further action by enzymes, but subsequent work by Wehrmann (110) demonstrated destruction by the bile. Buglia (16) was able to liberate the toxin from accompanying protein by enzyme action but with heavy loss of the toxin. Believing the previous data on this point to be

inconclusive, Buglia and Barbieri (18) made a further examination of the question and decided that the destruction was by acid rather than by enzymic action.

A peculiar burning taste is regarded as a property of the toxin, since the taste and the toxicity of preparations disappear simultaneously (69).

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