SYMPOSIUM ON NATURAL FOOD TOXICANTS

Introduction

This decade has witnessed an increasing and timely concern on the part of the public and their representatives over the possible harmful effects of chemical pollutants in our environment. Although it has not been popular to think in such terms, everyday food, too, is just a mixture of chemicals. In fact, eating represents the greatest direct exposure to exotic compounds that most of us ever received.

For centuries, people have been aware that certain plants and animals simply could not be used as food due to toxicity; in addition, some foods spoiled and became inedible, while others must be avoided at appropriate seasons. Only in recent years, however, have a number of more subtle diseases of men and domestic animals become associated with specific chemical constituents of food or forage.

Even the incidence of acute poisoning has been significant in terms of both public health and economics. Between 1956 and 1958, for example, nearly 500 persons lost their lives in Japan alone due to eating a type of poisonous fish; food and feed contaminated with ergot alkaloids altered the course of European history and later were responsible in considerable part for the establishment of the U.S. Department of Agriculture; and as much as 25% of newborn lambs in some flocks still may suffer abnormalities because of poisonous plants eaten once by their mothers during pregnancy. What, then, might we expect from longterm exposure to less spectacular toxicants in our common foods?

Several recent symposia and monographs have dealt with this subject primarily from the toxicological viewpoint. The present symposium for the Division of Agricultural and Food Chemistry was directed intentionally at the chemical aspects of these natural toxicants. The subject was divided arbitrarily into three parts to cover toxicants of animal, microbial, and higher plant origin, and I wish to thank my co-organizers F. E. Russell (University of Southern California) and Leo Goldblatt (U.S. Department of Agriculture, New Orleans) for their splendid cooperation in arranging the first two sections, respectively. Certainly, this has been the most extensive symposium ever held on the subject, and its publication, too, will represent a form of experiment. Through the cooperation of the American Chemical Society, the articles presented here will be reproduced by the Society in book form in order to reach the largest possible readership.

To my mind, the principal purpose of the symposium has been to introduce this fascinating subject to those who were not fully aware of its significance and, hopefully, to transmit to our audience some part of the enthusiasm of our panel of experts. If only a single advance in this field were to result from an interest generated here, the effort will have been more than justified.

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Studies on Shellfish Poisons

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Shellfish constitute an important part of the human diet. On occasion, shellfish have become poisonous and have caused paralysis and death. The poison originates in a marine dinoflagellate that occasionally grows in the water where the shellfish feed. It has been isolated from the hepatopancreas and siphon of shellfish, and is a heat-stable derivative of a purine base ($C_{10}H_{17}O_4N_7 \cdot 2HCl$). It is one of nature's most potent low molecular weight poisons. The poison also has been isolated from the dinoflagellate, *Gonyaulax catenella*, in axenic cultures and found identical to the poison isolated from the shellfish. Paralysis and death occur quickly, through inhibition of the sodium influx associated with a nerve impulse. No antidote is known.

Shellfish constitute one of the important seafoods for human consumption. Occasionally, in local areas, shellfish become extremely poisonous and have caused many outbreaks of food poisoning in humans resulting in sickness and death. The regions of the world where paralytic shellfish poisoning, sometimes called mussel poisoning, has occurred most often are around the North Sea, the north Atlantic coast of America, the north Pacific coast of America from central California to the Aleutian Islands, the coastal areas of Japan, and of South Africa. In general, these areas are 30° or more north or south latitude. The most recent outbreak of shellfish poisoning occurred during the latter part of this past June along the northeast coast of England. Because the shellfish poisons are not readily destroyed by heat processing or removed by washing of the shellfish, they present a unique public health problem for the commercial packers of shellfish. The peculiarity of the shellfish poison problem during the past 200 years was that the shellfish, which may have been

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good food for generations, suddenly, and for no apparent reason to the people at the time, would become extremely poisonous. The shellfish would remain so for one to three weeks and then, for no apparent reason, become safe for human consumption again.

This sporadic occurrence of poisonous shellfish was finally explained in 1937 when Sommer and Meyer (1937) and Sommer *et al.* (1937) and their coworkers at the University of California observed the presence of a particular microscopic plankton in the waters around the mussel beds during several outbreaks which occurred between 1920 and 1937 along the central California coast. These workers identified this organism as the dinoflagellate, *Gonyaulax catenella*, and found that it contained a poison that produced symptoms in mice similar to those of extracts of poisonous mussels. Their work showed that the mussels acquired the poisonous properties through the food chain and that they possess a mechanism in the dark gland or hepatopancreas that binds the poison. This dark gland usually contains 95% or more of the poison found in a mussel. Mussels gradually destroy or excrete the bound poison, so that within one to three weeks after the bloom of dinoflagellates has subsided, they are free of poison and safe for human consumption. However, in some clams, such as the Alaska butter clam, the poison apparently moves from the hepatopancreas to the siphon, where it is bound and may be retained for many months before decreasing to a safe level for human consumption. Generally, the physiology of the shellfish does not appear to be harmed by consuming the toxic dinoflagellate. The poison that is bound in the shellfish is readily released when they are consumed by man.

Several species of dinoflagellates are poisonous and have caused shellfish to become poisonous. The main ones reported in recent years are *G. catenella*, which has been found along the Pacific coast (Sommer and Meyer, 1937), *Gonyaulax acatenella*, along the coast of British Columbia (Prakash and Taylor, 1966), and *Gonyaulax tamarensis*, along the north Atlantic coast (Needler, 1949; Prakash, 1963). This organism was implicated as the cause of the recent outbreak of shellfish poisoning along the northeast coast of England (Wood, 1968; Robinson, 1968).

Whenever environmental conditions are right, these organisms grow out and may persist for one to three weeks in a particular area. The amount of poison in the shellfish depends upon the number of poisonous organisms in the water and the amount of water filtered by the shellfish. Along the California coast, mussels became too toxic for human consumption when 200 or more *G. catenella* cells were found per ml. of water. Low counts such as this can only be detected by microscopic examination of the water. This organism, like many others, will produce a visible "red tide" when the count reaches 20,000 or more per ml.

Because the poison does not effect the physiology of the shellfish, there are no distinguishing characteristics between poisonous and nonpoisonous specimens to serve as a guide to a person collecting them. The only practical means of detecting the poison is by a bioassay with mice. This assay is carried out on acidified water extracts (pH 2 to 3) of suspected shellfish. Serial dilutions of the extract are injected intraperitoneally into white mice weighing about 20 grams. The dilution that results in death in 15 minutes is defined as containing one mouse unit (Schantz *et al.*, 1958; Sommer *et al.*, 1937).

Symptoms of shellfish poisoning in man may be apparent within 30 minutes after eating poisonous shellfish. The symptoms begin with a numbness in the lips and fingertips, followed by progressive paralysis and death from respiratory failure in 2 to 12 hours, depending upon the magnitude of the dose. If a person survives 24 hours, the prognosis is good. Those who survive usually show no lasting effects from the ordeal. The dose to produce symptoms may be only a few hundred mouse units, and death may occur in humans from consuming 2000 to 3000 mouse units (Bond and Medcof, 1958). One small mussel weighing about 50 grams may contain several lethal doses, or as much as 20,000 to 30,000 mouse units. More specific details on shellfish poisoning are given by Sommer and Meyer (1937) and Schantz (1960).

The poison from poisonous California mussels and Alaska butter clams was isolated in pure form in 1954 after several years of a cooperative effort of workers at the University of California, Northwestern University, and the Army Biological Laboratories (Mold *et al.*, 1957; Riegel *et al.*, 1948; Schantz *et al.*, 1957; Sommer *et al.*, 1948). Poisonous mussels were collected along the Pacific coast near San Francisco and butter clams in southeastern Alaska. Acidified water extracts of the finely ground dark gland of the mussels were made, but in the case of the butter clams, extracts were made of the finely ground siphons. The purification of the poison in the extracts was accomplished by ion exchange chromatography on carboxylic acid resins, followed by chromatography on acid-washed alumina in absolute ethanol. By this technique, a white hygroscopic product was obtained that had a potency of 5500 mouse units per mg. of solids. Further chromatography employing a variety of techniques failed to improve the potency or change any of its chemical or physical properties. This fact was our basis for purity. The purified poison is a dibasic salt, pK_a at 8.2 and 11.5, and is very soluble in water. Its molecular formula as the hydrochloride salt is $C_{10}H_{17}O_4N_7 \cdot 2\ HCl$ (molecular weight 372). It has no ultraviolet absorption, gives positive Benedict-Behre and Jaffe tests, and is completely detoxified by mild catalytic reduction with the uptake of 1 mole of hydrogen per mole of poison at atmospheric pressure. Details of the purification procedure and the chemical, physical, and biological properties are described by Schantz *et al.* (1957, 1961) and Mold *et al.* (1957). Table I lists the important properties of the purified clam and mussel poisons.

The purified clam poison was studied by Rapoport *et al.* (1964) at the University of California, Berkeley; in 1964 they proposed an unusually substituted tetrahydropurine structure and named this substance "saxitoxin."

After the poison had been purified from toxic mussels and clams, studies were initiated to culture *G. catenella* and isolate the poison from this organism to compare it with the poison from clams and mussels. An acenic culture of *G. catenella* was obtained through the courtesy of Luigi Provasoli, Haskins Laboratory, New York, and used for these studies. This organism was cultured in sterile sea water contained in 2-liter Fernbach flasks and supplemented with small amounts of salts. After 17 days at 13° C., the cell count was about 30,000 per ml. The cells were filtered from the medium and lysed with dilute HCl at pH 2 to 3. This extract was processed through the carboxylic acid ion exchange resins and acid-washed alumina, exactly as the extracts of poisonous mussels and

Table I. Comparison of Properties of Poison from Cultured *G. catenella* Cells with Poison from Mussels and Clams

Property	Clam Poison	Mussel Poison	*G. Catenella* Poison
Toxicity	5500 MU/mg. ± 500	5500 MU/mg. ± 500	5010 MU/mg.
Specific optical rotation	130° ± 5° C.	130° ± 5° C.	127° C.
pK_a	8.3, 11.5	8.3, 11.5	8.2, 11.5
N content (Kjeldahl)	26.8	25.9	26.1
Sakaguchi test	Negative	Negative	Negative
Benedict-Behre test	Positive	Positive	Positive
Jaffe test	Positive	Positive	Positive
Absorption (220 to 360)	None	None	None
Infrared	3, 6, & 9 μ	3, 6, & 9 μ	3, 6, & 9 μ
Reduction with H_2	Nontoxic	Nontoxic	Nontoxic
Diffusion coefficient	4.9×10^{-6}	4.9×10^{-6}	4.8×10^{-6}

clams. The final product from this process was equal in toxicity to mice and identical in all of its chemical and physical properties to those of mussel and clam poisons. Table I gives a comparison of the properties of this poison to those of clams and mussels. The elemental analysis and molecular weight determination showed that the poison from *G. catenella* has the same molecular formula, $C_{10}H_{17}O_4N_7.2$ HCl. Details on the culturing, purification, and characterization of this poison are given by Schantz *et al.* (1966).

In an attempt to determine if there may be some differences in structure, Rapoport carried out degradation studies on each of the three poisons under identical conditions and compared the properties of each of the degradation products. Reduction with HI in glacial acetic acid and in the presence of red phosphorous yielded in each case 3-methyl-6,7-dihydro-5H-pyrrole-(1,2-C)pyrimidin-1-one. This product melted at 100–2° C. in each case and when mixed. Oxidation of the poison with alkaline hydrogen peroxide (1*M* NaOH, 1% H_2O_2) from each of the three sources yielded the same purine. Each degradation product was identical in ultraviolet and infrared absorption, in nuclear magnetic resonance, and in R_f values on paper chromatography. These data should leave little doubt that the poison from California mussels (primary source *G. catenella*), from the Alaska butter clams (primary source not known, Schantz and Magnusson, 1964), and from the *G. catenella* cells cultured in our laboratory, are identical substances.

These poisons are among the most potent known to man. In terms of the purified poison, one mouse unit, as defined previously, is equal to 0.18 μg. and is the weight of poison that produces death by intraperitoneal injection into a mouse weighing about 20 grams. The intravenous dose for a rabbit weighing 1 kg. is 3 to 4 μg. If it is assumed that 3000 mouse units is a lethal dose for man (estimated from accidental cases), then the weight of poison to cause death in man is 0.2 to 0.3 mg. by oral dose. In experimental animals, the oral dose is about 10 times the intraperitoneal dose. There is no known effective antidote for the poisons. However, the Klamath Indians used the gum from the sugar pine tree to overcome the toxic effects (Thompson, 1916). Studies thus far have not proven that the gum is really an effective antidote. Certain salts, such as sodium chloride, and alcohols, such as ethanol, reduce the effects of the poison to some degree. Because the diaphram muscles are particularly sensitive to the poison and death usually results from respiratory failure, artificial respiration has been effective in marginal cases.

These poisons have become of special interest to physiologists because they block the propagation of impulses in nerves and skeletal muscles without depolarization. Evans (1964) at the Sherrington School of Physiology, London, and Kao and Nishiyama (1965) at the New York State University found that the block is due to some specific interference with the increase in sodium permeability normally associated with excitation, and that the resting membrane conductances attributed chiefly to potassium and chloride permeabilities are unaffected. Sodium ions, therefore, do not diffuse into the cell to maintain the propagation of an impulse. The action is similar to that of tetrodotoxin from the puffer fish. Russel (1967) at the University of Southern California has compared the pharmacological action of many animal poisons and toxins, and has given further details on the action of shellfish poisons. Evans (1965) described in detail the cause of death in shellfish poisoning.

Besides being of value in physiological studies, the purified poison has served various governmental agencies as an international standard for the bioassay of commercial shellfish for human consumption. This is done to establish definite and rigid controls on the shipment of shellfish into and within the United States. Standard samples of purified shellfish poison for bioassay purposes can be obtained free of charge from Shellfish Sanitation, Department of Health, Education and Welfare, Washington, D. C.

Although progress has been made on the elucidation of the nature and properties of the shellfish poisons, many problems still remain that are important economically and academically. One of these problems concerns the poison occurring in the Alaska butter clam. These clams are one of the most palatable species known, but in some regions they are too poisonous for human consumption. These clams bind the poison in the siphon and it is held there for many months, even when removed to waters where the indigenous clams are not toxic. The fact that the chemical structure of the poison in these clams is identical to that of *G. catenella* might lead one to suspect this organism as the primary source of the poison, but this organism, or any other toxic organism, is seldom found in the Alaskan waters. Schantz and Magnusson (1964) found species of *Gonyaulax* in Alaska waters, but only on rare occasions and in extremely small numbers, and then not correlated with the presence of the poison in the clams. A means of controlling the poison in these clams would be of great economic value to Alaska. A study of the means by which shellfish bind the poison in their own systems might lead to a means of removing the poison from shellfish without destroying the commercial value. It seems evident that this problem in Alaska needs more study. Other studies of considerable interest would be the mechanism by which the various species of dinoflagellates synthesize the poison.

G. tamarensis produces a very potent poison that appears to be like *G. catenella* poison in its biological action, but indications are that it is somewhat different in its chemical and physical properties. This poison has not been isolated in pure form. An effective antidote is needed by the medical profession for the treatment of cases of poisoning. It is hoped, however, that the various studies on the mechanism of action will lead the way to an effective antidote. Preliminary studies have been carried out by Johnson *et al.* (1964) on the immunization of animals by using the poison as a haptene, and a small degree of protection has been obtained. Further studies along this line should be of value. Synthesis of the poison to verify the proposed structure is essential to our knowledge of its chemistry and to those studying its pharmacological action.

Other toxic organisms that may cause shellfish poisoning have been encountered in recent years and need further study. Ballantine and Abbott (1957) found *Gymnodinium veneficum* to contain a poison. Ray and Aldrich (1965), Spikes *et al.* (1968), and McFarren *et al.* (1965) found poisons in *Gymnodinium breve*. Gates and Wilson (1960) found a poison in *Gonyaulax monilata* and Schradie and Bliss (1962) reported poison in *Gonyaulax polyedra*. The poisons in these dinoflagellates are different in character than that from *G. catenella* and have not been implicated in human cases of shellfish poisoning (Halstead, 1965).

The trend toward the use of more products from the sea as food for man and animals makes it important that we know more about the various toxic organisms that might contaminate these food sources.

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