

A favorable milieu for the growth of staphylococcal enterotoxins in codfish may be created by extensive handling, use of certain salt concentrations, and long immersion in tapwater before cooking.

Two Poisoning Outbreaks in Puerto Rico From Salt Preserved Codfish

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IN THE FALL of 1957 Puerto Rican health authorities investigated a mass outbreak of food poisoning at the Ponce District Hospital and another at the institution for juvenile delinquents of San Juan. Both events were presumably caused by staphylococcal enterotoxins involving a common vehicle—salt-preserved codfish (Atlantic). The food in this form had not previously been incriminated in such intoxications.

The incident at the hospital, involving a

reported 303 patients in a total population of 457, occurred following lunch on September 5, 1957. There were no reports of acute gastroenteritis in the community outside the hospital. In the juvenile institution, the outbreak followed lunch on November 27, 1957, affecting 236 inmates out of a total of 320. Both outbreaks were first reported to local public health authorities, who in turn informed the bureau of communicable disease control in the Puerto Rico Department of Health.

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Ponce District Hospital Outbreak

Between the lunch at the Ponce District Hospital and the onset of illness, the mean duration of time was 3¼ hours, with a range of 1 to 15 hours. The onset was sudden, with vomiting, headache, and abdominal pain but no significant temperature change. The majority had diarrhea, and several noted mucus in the discharge.

The mean duration of illness was 6¼ hours, with a range of 2 to 24 hours. An associated fatality occurred in a chronically ill 53-year-old woman with long-standing pemphigus vulgaris, in whom postmortem examination revealed changes of the intestine compatible with acute inflammation.

The menu for the suspected lunch consisted

of codfish salad, tomatoes, starchy fruits and vegetables (plantains, yams, and sweet potatoes), bread, butter, whole milk, and guava paste. Each item was investigated as to manner of preparation, and an analysis of attack rates of illness was made with the result that all could be reasonably excluded as responsible vehicles with the exception of codfish salad (table 1).

Because of the small number of persons in the originally interviewed group that had abstained from codfish, all inpatients were questioned in an effort to include cardiac and diabetic patients who were served the same menu with the exception of codfish salad and guava paste. Only guava paste was included as a control food to expedite this second survey which covered 196 patients (table 2).

The high attack rate of gastroenteritis (79.2 percent) in patients eating codfish salad without guava paste and the absence of illness in 37 cardiac and diabetic patients who abstained from the salad strongly implicates codfish as the responsible item ($P < .0001$). The only cardiac patient who suffered symptoms admitted eating cod against dietary orders.

The entire hospital population is served from the single kitchen in which sanitation was found to be impeccable. The routine manner of preparing codfish salad was to allow whole fillets to desalt in cold tapwater for 12 hours during the night and to prepare the salad on the morning of serving. However, a significant modification in technique occurred on the morning prior to the outbreak, when the salted fish was

Table 2. Rate of attack of acute gastroenteritis among 196 patients eating certain food combinations for lunch at the Ponce District Hospital, September 5, 1957

Food combinations	Total persons	Number ill	Percent ill
Codfish salad, with guava paste.....	133	120	90.2
No codfish salad or guava paste.....	37	0	0
Codfish salad, no guava paste.....	24	19	79.2
Guava paste, no codfish salad.....	2	0	0

erroneously placed in hot tapwater. The average temperature of the kitchen is 100° F. during the day and 72° F. from 7 p.m. to 7 a.m. On the following morning the cod was prepared as a salad and distributed into thermatically controlled serving cars maintaining a temperature of 165° F. The food remained in these vehicles for 60 to 90 minutes before being consumed by the hospital population.

Five vomitus, two stool, and three blood cultures of severely ill patients revealed no staphylococci or other pathogens. The food bacteriology section of the health department could demonstrate no pathogens in any of the food items after considerable effort. Two frozen samples of cod were referred to the bacteriology department of the University of Puerto Rico Medical School after several weeks, because of the epidemiological implication. There, a coagulase-negative strain of *Staphylococcus*

Table 1. Rate of attack of acute gastroenteritis among persons eating lunch, Ponce District Hospital, September 5, 1957

Food item	Persons eating food item			Persons not eating food item		
	Total number	Number ill	Percent ill	Total number	Number ill	Percent ill
Codfish salad.....	289	254	87.8	5	3	60.0
Tomatoes.....	141	120	85.1	153	137	89.5
Yams.....	282	246	87.2	12	11	91.6
Sweet potatoes.....	287	251	87.4	7	6	85.7
Plantains.....	285	250	87.7	9	7	77.8
Bread.....	205	173	84.4	89	84	94.3
Butter.....	108	92	85.2	186	165	88.7
Whole milk.....	266	230	86.4	28	27	96.4
Guava paste.....	266	232	87.2	28	25	89.3

pyogenes var. *aureus* was readily demonstrated in both specimens in the approximate concentration of 10^6 organisms per gram. This strain was subsequently encountered by the food bacteriology section of the department and has been confirmed as such by the Staphylococcal Unit of the Communicable Disease Center, which undertook phage typing of strains isolated in the outbreaks.

No cultural examination of the kitchen personnel was made for staphylococcal carriers because of the original lack of success in isolating this organism from the food. There was, however, no evidence of superficial purulent lesions or a history of recent upper respiratory infection.

Outbreak at the Juvenile Institution

In the outbreak at the institution for juvenile delinquents, the mean interval between lunch and the onset of illness was slightly less than 2 hours, with a range of several minutes to 8 hours. The symptoms were similar to those of the previous outbreak, and the duration of illness was less than 24 hours in two-thirds of the cases and from 1 to 4 days in the remainder.

The menu consisted of codfish salad, rice and beans, bread pudding, and bread. An analysis of attack rates of illness by food consumed by 223 available inmates (table 3) strongly implicates codfish salad as the responsible item ($P < .0001$). Furthermore, an analysis of attack rates by food combinations reveals an absence of illness in inmates consuming either rice and beans or pudding but abstaining from the salad (table 4).

The preparation of codfish salad at this institution left no doubt as to its potentialities to

Table 4. Rate of attack of acute gastroenteritis among persons eating certain food combinations for lunch at the institution for delinquents, November 27, 1957

Food combinations	Total persons	Number ill	Percent ill
Rice and beans.....	221	177	80.1
With pudding.....	193	158	81.9
Without pudding.....	28	19	67.9
With codfish.....	203	177	87.2
Without codfish.....	18	0	0
Pudding.....	194	159	81.9
Without rice.....	1	1	100.0
With codfish.....	184	159	86.4
Without codfish.....	10	0	0
Codfish.....	205	179	87.3
Without pudding.....	21	20	95.2
Without rice.....	2	2	100.0
Total persons eating..	223	179	80.3

support the production of enterotoxin. Food for the inmates is prepared in a separate kitchen, which is of substandard hygiene and is staffed by the inmates. At 3:00 p.m. in the afternoon, 20 hours before serving, 100 pounds of cod were boiled for 1 hour. Fresh tapwater was added to the caldron and three inmates extracted fish by hand to remove bones. At 5:30 p.m., with one-quarter deboned, the work was abandoned to be completed the following morning. The fish remained at room temperature until 11:30 a.m., when it was served after light seasoning.

No specimens were obtained from patients. The food bacteriology section isolated various strains of *Staphylococcus pyogenes* from the rice and bread pudding but could not demonstrate any such contamination in the salad. However, the school of medicine isolated a coagulase-positive strain of *Staphylococcus*

Table 3. Rate of attack of acute gastroenteritis among persons eating lunch at the institution for delinquents, November 27, 1957

Food item	Persons eating food item			Persons not eating food item		
	Total number	Number ill	Percent ill	Total number	Number ill	Percent ill
Rice and beans.....	221	177	80.1	2	2	100.0
Pudding.....	194	159	81.5	29	20	69.0
Codfish salad.....	205	179	87.3	18	0	0
Bread.....	202	165	81.7	21	14	66.7

pyogenes var. *aureus* in the codfish salad with an estimated concentration of 1.6×10^7 organisms per gram. All strains were confirmed by the Staphylococcal Unit of the Communicable Disease Center. The one isolated from the cod and two other coagulase-positive strains from rice and bread pudding proved resistant to the phage types used. Ten of the twelve food handlers were also shown to carry coagulase-positive *Staphylococcus pyogenes* var. *aureus* including six phage-resistant strains.

Enterotoxin Production

Isolated strains of staphylococci from the codfish salad served at the hospital and the institution for juvenile delinquents were referred to the Robert A. Taft Sanitary Engineering Center, Public Health Service, for enterotoxin production experiments.

Heated culture filtrates were tested by intravenous injection into cats. None of the laboratory animals receiving the filtrates prepared from the cultures gave the response which typically follows the injection of enterotoxin.

According to Dr. Foter, who supervised the enterotoxin testing at the Robert A. Taft Sanitary Engineering Center, there is at present no completely satisfactory experimental test for enterotoxin. The most reliable test is the feeding of suspected food to human volunteers, which is obviously impractical as a routine test.

The feeding of laboratory animals with suspected food results in nonspecific responses. In Dr. Foter's opinion, intravenous injection of a heated culture filtrate of staphylococci isolated from food into cats has limited value. The observations indicate that a positive response would be reliable, but the meaning of a negative response is not clearly understood.

Also, there is evidence that the laboratory animals used for this type of test, the monkey and the cat, are less susceptible to enterotoxin than man. An assay which can be performed quickly with simple equipment and with a high degree of reliability is urgently needed.

Discussion

The success of the medical school laboratory in isolating staphylococci from the codfish

salad was attributed to the use of milk with 10 percent sodium chloride agar for plating after making appropriate dilutions. This type of selective medium was not used by the food bacteriology section of the health department. *Staphylococcus pyogenes* has been shown to have a high salt tolerance (1), with enterotoxigenic strains proved capable of initiating growth in a 20 percent sodium chloride broth (2). Nutrient media containing 7.5 to 10 percent sodium chloride, in fact, have previously been demonstrated to be preferable for the isolation of *Staphylococcus pyogenes* from sources highly contaminated with other organisms (3,4).

To date there has been no demonstration that naturally occurring coagulase-negative *Staphylococcus pyogenes* produces enterotoxin (2,5,6), but the strain isolated in the outbreak at the Ponce District Hospital (from 4 different food samples by 2 laboratories) bears a suspicious relation to the etiology, and we believe it merits further study.

The time, temperature, and salt-concentration conditions that prevailed in the preparation of the salad prior to the Ponce hospital outbreak were duplicated in the food bacteriology laboratory. The extended desalting allowed luxuriant growth of staphylococci, but the seasoning and heating process before serving resulted in a marked diminution of these organisms. Therefore, a possibility to be considered is that an enterotoxin may have been produced by a strain of staphylococcus which was destroyed by final heating, and that the coagulase-negative strain appeared as a contaminant. It is unlikely that any of the temperature factors, detrimental as they may have been to these organisms, would have altered a formed toxin (7).

Phage typing of enterotoxin-producing strains of coagulase-positive staphylococci has received attention (8-10). Although it has been found that the great majority of the reported strains belonged to a reasonably easily defined group of phage patterns, approximately 12 percent were phage resistant, as was the case with the strain isolated from the codfish salad implicated in the outbreak at the juvenile delinquents' institution.

A brief review of the dry-salting process of

preserving codfish is pertinent to an understanding of the food's potentialities for contamination with enterotoxigenic staphylococci. The flesh and body cavities of salt water fish are sterile (11), but the surfaces and gut may be contaminated in the order of 10^2 to 10^7 . Dyer has demonstrated that 73 percent of aerobic flora of Atlantic cod may belong to the micrococcus group and that 7 percent of these were *Staphylococcus pyogenes* vars. *aureus* and *albus* (12). However, these organisms appeared to be marine in origin (13) and were not considered potentially pathogenic because of a uniformly negative coagulase reaction (14). When the fish arrives at shore and is handled, the micrococcus flora increases in inverse ratio to the degree of cleanliness of the premises (15). Of the micrococci growing at 98.6° F., 10 to 16 percent were shown to be indistinguishable from coagulase-positive *Staphylococcus pyogenes* var. *aureus*. These micrococci must be considered potentially pathogenic (16).

After filleting, the cod is preserved with salt which appears to be a complex process involving the inactivation of bacterial enzymes besides the withdrawal of moisture, thus restricting microbiological activity (17). Salt is interspersed between layers of split fish, establishing a concentration of about 20 percent in 14 to 16 days while the juices are allowed to run away, with a maximum loss of weight of 30 percent (18). The flesh, at this stage "wet stack" or "green" cured, always contains some microorganisms, almost exclusively micrococci, and never becomes sterile (15). Spoilage at this stage is known as "pinkening" because of the characteristic pigment produced by the contaminating flora (19). Food poisoning was observed in association with spoiled and "pink" cod as early as 1886 (20), but the nature of the poisoning has not been clearly defined.

The fish is then hard dried by exposure to wind and sun and is no longer susceptible to such contamination unless stored in relative humidities of 75 percent and greater. Boury (21) has demonstrated, however, that *Staphylococcus pyogenes* var. *aureus* may survive in hard-dried cod fillets and even grow under suitable conditions of humidity.

This report has demonstrated the hazard of exposing salted cod to water at environmental

temperatures for extended periods of time. Although a preformed staphylococcal enterotoxin has not yet been demonstrated in salted cod during its processing or storage, the logical extension of available evidence forces one to consider this a strong possibility. More adequate information, therefore, is needed with regard to the safe hygienic standards in these phases for the protection of the public's health.

The study also illustrates the need for careful analysis of information obtained from field investigations of foodborne outbreaks, inasmuch as definite laboratory confirmation of causative factors often is not obtainable.

Summary and Conclusions

Two food poisoning outbreaks in Puerto Rico during the fall of 1957 were ostensibly caused by staphylococcal enterotoxin in salt-preserved codfish, a product that had not previously been incriminated in such mass intoxications.

The first outbreak, at the Ponce District Hospital, affected 303 persons and the second, 236 inmates of a juvenile delinquents' institution. Laboratory tests on samples of the codfish salad served in the second outbreak yielded strains of *Staphylococcus pyogenes* var. *aureus* when a 10 percent sodium chloride nutrient medium was used.

The findings support the view that partial preservation of codfish by salting does not completely destroy contaminating organisms which have a high salt tolerance. In fact, the addition of suitable concentrations of salt may serve to kill spoilage bacteria which might compete for growth with the staphylococcus.

These food poisoning experiences demonstrated the hazard of consuming salt-preserved codfish following its extended exposure to water at environmental temperature.

Enterotoxin production studies were carried out on cats in the laboratories of the Robert A. Taft Sanitary Engineering Center. When the animals received filtrates from cultures of strains isolated in the outbreaks, they did not give the response which typically follows injection of enterotoxin.

This type of test, however, results in non-specific responses. There is evidence, more-

over, that both the monkey and the cat are less susceptible to enterotoxin than man. There is urgent need for a simple, fast, but reliable test for enterotoxin.

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