Botulism From Canned Tuna Fish

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IN MARCH 1963, three Detroit women were clinically diagnosed as victims of botulism following a luncheon which included tuna fish salad sandwiches. Two of them died. Subsequent epidemiologic and laboratory findings incriminated *Clostridium botulinum*, type E, in commercially canned tuna fish as the causative organism.

The Wayne County (Mich.) Health Department alerted the Detroit District Food and Drug Office, on March 15, to a death believed to be due to botulism and to the hospitalization of a woman with symptoms of botulism.

Epidemiologic investigation by two Food and Drug Administration inspectors revealed that on March 14, about 11 a.m., the deceased, Mrs. B; her hospitalized neighbor, Mrs. M, who subsequently died; and her mother, Mrs. K, who became ill, had luncheon at Mrs. B's home. The meal consisted of tuna fish salad sandwiches, vegetable soup, and coffee. Mrs. B had eaten a sandwich, soup, and coffee. Mrs. M had only a sandwich and coffee. Mrs. K joined them a little later and ate the small portion of tuna salad that remained, as well as soup and coffee.

Mrs. K stated later that the salad contained only two ingredients: canned tuna fish and salad dressing. Allegedly, while preparing the salad, Mrs. B had questioned the odor of the tuna, but Mrs. M was unable to detect an abnormal odor. Both had tasted the tuna and decided that it was "all right."

While eating dinner at home, about 6 p.m., Mrs. B complained of blurred vision, and she repeatedly took off and put on her eyeglasses. At 7:30 she went to bed complaining of difficulty in breathing and a tightness in her throat, as well as the vision difficulty. The next morning, at 6:30, Mrs. B was suffering convulsive respiration and could speak only in a whisper. She was sent to the hospital in an ambulance, but she was dead on arrival at 7:30 a.m.

Mrs. M became ill about 7:30 p.m. following the luncheon. She complained of dizziness, blurred vision, and difficulty in breathing. Later her movements became somewhat uncoordinated and she vomited frequently during the night. Mrs. M was hospitalized at 8 a.m. and given polyvalent types A and B botulinus antitoxin. Her symptoms continued to progress. On the fourth day after the luncheon she was given type E botulinus antitoxin, but she did not improve. She died at 5 p.m. on March 19.

Mrs. K suffered nausea and vomiting about 24 hours after eating the small portion of tuna salad, and she was hospitalized. She complained of a sore throat and she had some vision difficulties. Mrs. K was given 10,000 units of polyvalent types A and B botulinus antitoxin. She made a relatively rapid recovery, and she was released from the hospital 3 days later.

The illness of the three women was diagnosed as botulism. They had eaten three items in common: coffee, bread, and tuna fish salad. About 5 percent of the dressing used in the salad was left in the 1-quart jar. Since a relatively small amount of salad dressing is needed for 6½ ounces of tuna fish, the dressing presumably had been used previously without ill effects.

Samples from the garbage can at Mrs. B's home were delivered to the laboratory on March 16. From the case investigation, it was known that the can had been emptied on the morning of the luncheon. The samples consisted of three newspaper-wrapped packages of miscellaneous garbage, two empty soup cans, and a tuna can

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lid with code impressions. Also picked up from Mrs. B's home were two intact cans of cut green beans, an intact can of tuna fish, an intact jar of salad dressing, and a wall-type can opener. The empty can which had contained the suspect tuna and the dressing used to prepare the salad had been delivered previously to the health department by the local police. These two samples and Mrs. B's stomach and intestinal contents, removed at autopsy, were obtained later by the laboratory. In addition, 16 cans of tuna and 6 quarts and 3 pints of salad dressing were picked up from the shelf stock of the store where Mrs. B had purchased the suspect items. All the jars of salad dressing and six of the tuna cans bore the same code as those used to prepare the salad.

Laboratory Studies

The intact food containers were opened aseptically and portions of the contents were inoculated into tubes of cooked meat media and streaked on liver-veal agar plates containing 0.005 percent neutral red (*1*). Swab specimens from the empty cans, can lids, can opener, and garbage materials were also inoculated into tubes of cooked meat media and streaked on liver-veal agar plates containing neutral red. Multiple tubes and plates were incubated at both 30° C. and 32° C. Tubes or plates showing growth were streaked on McClung-Toabe egg-yolk agar.

Clostridial forms were observed in the cultures of swab specimens from the lid and can of the suspect tuna, the stomach and intestinal contents of Mrs. B, and the dressing used to prepare the salad.

Cooked meat cultures of the specimens from the lid of the tuna can, salad dressing, and stomach contents were incubated for 16 hours and filtered through cotton. The filtrates, unheated or heated for 10 minutes at 100° C., were injected intraperitoneally into mice. Also, portions of the unheated filtrates were injected into mice protected with 0.2 cc. monovalent types A and B botulinus antitoxin. Only those mice challenged with the boiled filtrates survived. The results of these mouse toxicity tests with 16-hour impure cultures from the tuna can lid, salad dressing, and stomach contents were as follows:

Filtrates (0.8 cc.)	*Results*
Unheated	Fatal
Heated	Nonfatal
Unheated +0.2 cc. type A antitoxin	Fatal
Unheated +0.2 cc. type B antitoxin	Fatal

After 72 hours' incubation, the same cultures were diluted 1:10 with normal saline and mouse toxicity tests were repeated, as described above, with the addition of monovalent type E botulinus antitoxin. The results indicated that these 3-day impure cultures from the tuna can lid, salad dressing, and stomach contents contained type E botulinus toxin, as shown below:

Filtrates (0.5 cc. of 1:10)	*Results*
Unheated	Fatal
Heated	Nonfatal
Unheated +0.2 cc. type A antitoxin	Fatal
Unheated +0.2 cc. type B antitoxin	Fatal
Unheated +0.2 cc. type E antitoxin	Nonfatal

Residual toxicity assays were performed on the stomach and intestinal contents obtained on autopsy, the salad dressing, and the rinsings from the suspect tuna can. The stomach and intestinal contents and the salad dressing were diluted 1:5 with normal saline, 0.1 percent trypsin was added, and the mixtures were incubated for 1 hour at 35° C. (*2*). The mixtures were centrifuged, and 0.5 cc. of the supernatant was injected intraperitoneally into mice. The suspect tuna can was obtained 5 days after the luncheon from Dr. Rosenbaum of the Wayne County Health Department. Dr. Rosenbaum had rinsed the can with saline and injected the rinsings intramuscularly into a mouse, which died within 36 hours. In our laboratory, the can was rerinsed with 4 cc. of normal saline. The rinsings were trypsinized, centrifuged, and injected into mice. None of the materials assayed exhibited any residual toxicity.

Approximately 2 ml. of the original saline rinsings were obtained 7 days after they were prepared. These rinsings were trypsinized, as described previously, and injected intraperitoneally into mice. The results, although inconclusive, indicated that the can rinsings contained a small amount of type E botulinus toxin, as shown below:

Rinsings (0.5 cc.)	*Results*
Unheated	Nonfatal
Heated	Nonfatal
Unheated +0.2 cc. type A antitoxin	Fatal in 16 hours
Unheated +0.2 cc. type B antitoxin	Fatal in 16 hours
Unheated +0.2 cc. type E antitoxin	Nonfatal

Pure culture isolates were obtained from the tuna can lid and the salad dressing. These isolates were grown in TPG broth for 4 days at 30° C., diluted 1:5 with normal saline, and trypsinized. Sterile culture filtrates, obtained by filtering through Seitz filters, were injected intraperitoneally into protected and unprotected mice. Only the mice protected with 0.2 cc. of type E antitoxin or injected with the heated, pure-culture filtrates from the lid and salad dressing survived, as shown below:

Filtrate (0.4 cc. of 1:5)	*Results*
Unheated	Fatal
Heated	Nonfatal
Unheated +0.2 cc. type A antitoxin	Fatal
Unheated +0.2 cc. type B antitoxin	Fatal
Unheated +0.2 cc. type E antitoxin	Nonfatal

The intestinal and stomach contents were heavily contaminated with both aerobic and anaerobic spore-forming bacteria. *C. botulinum*, type E, in pure culture was isolated from the contents of the stomach but not from those of the intestinal tract.

Approximately 1,200 additional cans of the same brand of tuna were classified as to code and condition. Specimens from six of these cans, which were found to be leaking, were cultured. Bacteria other than the clostridial forms were found. Two cans classified as flippers were dry-packed and sterile. One swollen can, bearing a code different from that of the incriminated can, contained an apparently pure culture of a clostridial form which was morphologically and culturally similar to the *C. botulinum* isolates. In mouse toxicity tests, however, neither its contents nor 9-day cultures of the isolates were toxic to the mice. This can had been poorly seamed and all its packing fluids had leaked out.

Discussion

Clinical, epidemiologic, and laboratory findings indicated that the three cases of botulism were caused by *C. botulinum*, type E, in tuna fish. This tuna was received frozen from Japan and commercially canned in the United States. This was the first outbreak of botulism from U.S. commercially canned products in 40 years. One other can from the same packer and of the same code was found by another laboratory to be contaminated with *C. botulinum*, type E.

Tuna Fish Cans Defective

March and April 1963 will be remembered as "tuna fish months" by many health officials throughout the nation. Particularly involved were health departments in Michigan, California, and New York.

On March 15, the Wayne County (Mich.) Health Department notified the Detroit District of the Food and Drug Administration concerning three cases of botulism, in which two fatalities resulted. These are still the only known instances of botulinus poisoning attributed to canned tuna.

On April 12, the California State Department of Public Health issued a quarantine order closing the cannery which packed the tuna. On the same date, the New York City Health Department embargoed all lots of Dagim Tahorin brand of kosher tuna from the cannery, after cans of this pack had been found by FDA bacteriologists to be contaminated. City, State, and Federal inspectors worked with the distributors to recover the product from retail outlets.

Throughout the country, health departments collaborated with FDA district offices in answering consumer inquiries and checking on suspect cans reported or turned in by retailers and the public.

At press time, a total of 21 cans of tuna containing the botulinus organism had been found by FDA bacteriologists. Most such cans had defective seams, indicating that the organisms entered the cans after sterilization had taken place.

The salad dressing was normal in odor and appearance, and it had a pH value of 3.6. By direct smear, it contained few bacterial cells per field; however, long, vacuolated gram-positive rods containing enlarged oval spores in a terminal to subterminal position typical of *C. botulinum* were seen. The presence of the organism in the remaining salad dressing was assumed to be a result of using the same spoon to scoop the tuna from the can into a dish and to remove the salad dressing. The only survivor of the luncheon was not present when the salad was being prepared, and the two victims were too ill to be interviewed before they died.

Coffee and bread also had been consumed by all three women. The coffee was from a 3-pound package that had been used before and

after the luncheon with no ill effects to other members of the family. The bread was part of a commercially baked loaf that also had been used before and after the luncheon with no ill effects.

Isolates from the suspect items were weakly toxigenic, a condition not uncommon with primary isolates of *C. botulinum*, type E. Young cultures could be typed, however, with the use of undiluted, unfiltered preparations. In these tests, type E antitoxin protected mice, whereas types A and B did not. When trypsinized, 3- to 4-day-old cultures could be diluted 1:5 or 1:10, Seitz filtered, and typed. Since the tuna had been in the can for 2 months before it was used, it is conceivable that cultures in broth for this length of time would be more toxic. The tuna can and lid had no holes or seam leaks.

Summary

In March 1963, three women were clinically diagnosed as victims of botulism following a luncheon which included tuna fish salad sandwiches. The salad had been freshly prepared from canned tuna which allegedly had an abnormal odor when it was opened.

One woman developed severe symptoms 7 hours after the luncheon, but she did not vomit. She died within 21 hours. The second victim developed severe symptoms 8 to 9 hours after eating the salad, vomited frequently, and died 5 days later. The third woman, who had eaten a small portion of the tuna salad, developed mild symptoms within 24 hours. She recovered 3 to 4 days later.

Clostridium botulinum, type E, was recovered from the empty tuna can and lid, the stomach content of one victim, and the remaining portion of salad dressing used to prepare the salad.

REFERENCES

(*1*) Kaufman, L., and Weaver, R. H.: Use of neutral red fluorescence for the identification of colonies of clostridia. J Bact 79: 292–294 (1959).

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Study of Discrimination in Health Facilities

As part of a nationwide survey of the policies and practices of health services and facilities in respect to race, the U.S. Commission on Civil Rights has sent a special questionnaire to a random sample of 400 hospitals, mainly in medium-sized counties having a nonwhite population of 5,000 or more.

Investigation of racial discrimination is also an important part of the Commission's program of urban area studies, which currently includes Newark, Memphis, Indianapolis, Chicago, Los Angeles, and Washington, D.C. The Commission issues reports on each city and transcripts of public hearings held.

In addition, several of the State advisory committees to the Commission are studying racial discrimination in the health facilities and services of their States, assisted by the Commission upon request. Results of one study by a State committee are reported in a publication entitled "Equal Protection of the Laws in North Carolina," on sale for $1 at the Government Printing Office, Washington 25, D.C.