General features of the species

*Clostridium botulinum* is one of many gram positive, strictly anaerobic spore forming rods. It shares with *Clostridium perfringens*, *Clostridium tetani*, and several other species the ability to produce extremely potent and highly antigenic exotoxins that are harmful to human tissues.

The toxin of *C. botulinum* is a protein. Man acquires it by eating food in which the organism has grown. Inadequately processed home canned vegetables are the usual vehicle of poisoning in this country, but meat products are more common in Europe. Lightly salted, smoked, partly dried, pickled, or fermented fish also have been incriminated in some parts of the world.

Botulinum toxin acts on the nervous system by interfering with the passage of nerve impulses. Symptoms usually appear within 12 to 36 hours after the food is consumed. Nausea and vomiting may be evident first, followed by neurologic symptoms such as double vision, muscular weakness, and difficulty in talking and swallowing. Death, which occurs in about one-third to two-thirds of the cases, results from respiratory paralysis.

Botulism, or sausage poisoning as it was originally called, has been known for more than two centuries, but the causal organism was isolated only 70 years ago. Subsequent investigations have demonstrated at least six distinct types of *C. botulinum* based on the serological specificity of their toxins. These types are designated by the letters A through F. Presumably man is susceptible to all six types, although most of the outbreaks have been attributed to types A, B, and E. Types C and D usually are associated with botulism in animals. Type F was discovered in Denmark in 1958 as the cause of an outbreak traced to home prepared liver paste (Dolman and Mirakami, 1961). Thus far no other outbreaks have been attributed to this type.

**Characteristics of type E**

The first known outbreak of type E botulism occurred in New York State in 1932 following the consumption of smoked salmon imported from Labrador. The causal organism was isolated but was not identifiable with the antisera then available. Subsequently, toxigenic cultures resembling *C. botulinum*, but also unidentifiable with types A, B, C or D antisera, were isolated from fish in Russia. In 1936 these cultures were designated as type E by Dr. K. F. Meyer and his associates in California. Then it was recognized that the culture isolated four years earlier in New York State
belonged to this new serological type. Thus almost 40 years elapsed between
the original isolation of C. botulinum from ham in 1895 and the discovery
of type E in a fish product.

Type E botulism is clinically identical to that caused by the
more common types A and B, but the organism and its toxin have several dis-
tinctive and significant features. Perhaps the most remarkable character-
istic is the low heat resistance of type E spores. Although these struc-
tures appear to be perfectly normal and show typical refractility under the
phase microscope, their heat resistance, according to Ohye and Scott (1957)
is only about 1/1000 that of types A and B.

It has long been known that spores of types A and B can withstand
boiling for five or six hours. Spores of type E, on the other hand, are
rapidly killed at temperatures as low as 80°C. To cite several examples,
Gunnison, Cummings and Meyer (1936) found that 5 million type E spores per
ml of pH 7.4 buffer were destroyed after 2 minutes at 100°C. or 6 minutes
at 80°C. Pederson (1955) and Hazen (1937) reported that their cultures
withstood only 10 minutes at 80°C. Nakamura et al (1956) observed complete
destruction of eight strains of type E in saline after 20 minutes at 80°C.
or 5 minutes at 90°C.

In more refined experiments recent workers have shown the loga-
rithmic death rate of several type E strains to be in the range of 0.5 to
3.5 minutes at 80°C. when the spores were heated in broth, distilled water,
physiological saline, or phosphate buffer. Thus there is no doubt that the
majority of type E spores are indeed highly sensitive to heat. In all
likelihood it was this property that delayed so long the isolation of the
organism. This statement will be readily understood when it is remembered
that most of the early workers routinely heated samples of soil and other
natural materials to 80°C. for 10 minutes when testing for Clostridium
botulinum.

Before we eliminate type E from further consideration in heated
products, however, we should remember that there have been a few exceptions.
Dolman and Chang (1953) reported that a few spores occasionally withstood
100°C. for 30 minutes, and Graikoski and Kempe (1964) found small numbers of
survivors after heating at 85°C. for two hours or 90°C. for one hour. Sev-
eral other workers occasionally have encountered type E spores with un-
usually high thermal resistance.

It is evident, as Schmidt has pointed out (1964), "that far too
little is known about the thermal resistance of type E spores. The resist-
ance of most populations may be low, but the occasional occurrence of spores
of much higher resistance cannot be excluded". We especially need to know
more about the heat resistance of different strains and about the effect of
the heating and the recovery media on their resistance. We can by no means
be sure that thermal resistance data obtained in phosphate buffer, for ex-
ample, will be applicable to the heat treatment of natural products such as
fish.

A second distinguishing feature of C. botulinum type E is its low
minimum temperature for growth. Dolman et al (1950) were the first to ob-
serve this property when they detected growth and toxin production by their
VH strain at 6°C. Later, Ohye and Scott (1957) recorded growth of several cultures at 5°C. In 1961 Schmidt, Lechowich and Polinazzo reported growth of four strains in a beef stew medium at 3.3°C (38°F). The time required for visible gas production at this temperature was 31 to 45 days. This finding has been confirmed by Kempe and Graikoski (Kempe, 1965), who observed growth of type E in irradiated haddock within 12 days at 37°F.

Thus it appears that the minimum temperature for growth of C. botulinum type E is approximately 10 to 12°F lower than that for types A and B. The significance of these findings is obvious. If viable type E spores are present in a suitable foodstuff they may germinate, grow and produce toxin at temperatures that are normally considered to be good refrigeration; that is, 37° or 38°F.

A third distinguishing feature of type E is its relatively low salt tolerance in comparison with types A and B. Although very little has been published on this subject, personal communications from several laboratories indicate that the limiting concentration of salt is in the neighborhood of 5%. For types A and B, on the other hand, 8.0 to 10.0% salt is generally considered to be necessary to prevent growth and toxin formation (Greenberg, Silliker and Fatta, 1959). If further work proves that 5% salt is, in fact, near the limiting concentration, we shall have a very important safety factor for some foods. It should be remembered that the critical factor is the concentration of salt in the aqueous phase of the food. Thus, partial drying has the same net effect as adding more salt.

A fourth and most interesting characteristic of type E is its relatively low toxicity as measured by titration in mice. Most cultures prove to have about 1,000 to 2,000 mouse lethal doses per ml, and it is the unusual one that contains 5,000 MLD. Cultures of type A, on the other hand, regularly contain 100,000 to 1,000,000 mouse MLD per ml, and those of type B are only slightly lower as a rule.

For a long time the relatively low toxicity of type E cultures could not be correlated with the worldwide case fatality rate of about 30% (Dolman, 1964), which approaches the fatality rate for type A. Then in 1956 Duff, Wright and Yarinsky discovered that the toxicity of type E cultures could be increased 10 to 50 fold by incubating the toxic supernatant with trypsin. Japanese workers have described similar "activation" of type E toxin by proteolytic enzymes of bacterial origin. These observations have given rise to the suggestion that type E produces a "protoxin", which can be "activated" by proteolytic enzymes. They may also explain the relatively high toxicity of type E cultures for man as compared with that for mice. It has been suggested that the protoxin is activated by the proteolytic enzymes in man's digestive tract after the toxin is swallowed.

The relatively high initial toxicity of type A cultures is explained on the basis that this organism produces its own proteolytic enzymes, which presumably activate the toxin before it is released from the cells. Type E, on the other hand, is not proteolytic and therefore, according to current ideas, releases unactivated protoxin.

A fifth characteristic of C. botulinum type E is its almost invariable association with fish or other animal products from an aquatic
environment. Moreover, the disease usually is caused only by foods that are processed in some way, as by mild heating, fermenting, pickling, smoking, "curing", and the like. Table 1 shows the worldwide distribution of the known outbreaks of type E through 1963. The great majority of the Japanese incidents have been caused by izushi, a dish prepared in the home by packing raw fish, rice, diced vegetables and a little salt into a wooden tub and allowing the mixture to ferment for 3 or 4 weeks at room temperature. Most of the Canadian outbreaks were traced to pickled herring, fermented or putrefied salmon eggs, or cured seal meat. The latter two preparations are frequently eaten by native Indians and Eskimos. Pickled fish have been involved in most of the outbreaks in the Scandinavian countries, while "salted, dried and cold-smoked fish" have caused outbreaks in the Soviet Union.

Table 1 lists all known outbreaks of type E in the United States. Whale meat and salmon eggs have been the usual cause in Alaska, whereas smoked fish has been the most common vehicle in the remainder of the country. Two outbreaks were traced to canned fish. In one of these the cans were shown to be improperly sealed.

Table 2 gives one example of a food that did not come from an aquatic environment—mushrooms imported from Yugoslavia and canned in California. Dolman (1964) has cited two other examples, both from Russia; these were home-pickled ham and pickled red beets. No information was given to suggest how these products became contaminated.

The association of C. botulinum type E with marine animals and the failure to find it in surveys of inland soils gave rise to the belief that it is indigenous to the sea. More recently, however, Dolman (1957) has suggested that type E is a terrestrial organism whose spores are carried to the sea by run-off water from the land. He further suggests that type E exists in local concentrations, irregularly dispersed over the earth, which may account for the occurrence of type E botulism in some places and not in others.

Other workers have substantiated Dolman's terrestrial theory. In extensive surveys, Kanzawa (1960) and other Japanese workers have found C. botulinum type E in the soil and mud throughout Hokkaido Prefecture in Japan. Johanssen (1963a) has isolated it repeatedly from samples taken in Sweden, and has found it on potatoes grown in several countries. Thus it is clear that the organism occurs at some distance from the sea.

Johanssen also has found type E frequently both in bottom sediments and along the shore line of the Baltic Sea. In the Oresund, which separates Sweden from Denmark, he has found the organism in practically every sample of bottom sediment tested. Johanssen explains his findings on the basis that the Baltic Sea is a catchment basin for a large European land mass from which the spores of type E are carried by run-off water.

One large area in which type E has not been demonstrated is the British Isles. Extensive studies there during the past two years have not revealed a single culture of the organism from British soil or fish (Hobbs, 1965). Furthermore, type E appears to be rare in the North Sea.
In 1960, an outbreak of type E botulism traced to smoked fish from one of the Great Lakes suggested that the organism may exist in mid-continental areas. Consequently, a survey of the Great Lakes was begun in our laboratory during the latter part of 1963. We have used the intestinal contents of fish as the chief test material, although samples of water and mud also have been examined. Table 3 summarizes our results thus far with fish.

It is now clear that C. botulinum type E may be found in all four of the Great Lakes studied, although its relative incidence varies greatly. It was rare in the fish of Lakes Erie and Superior, more frequent in Lake Huron, still more frequent in Lake Michigan, and abundant in the fish of Green Bay. We have had no difficulty finding the organism in the mud and water of Green Bay, although our tests have rarely demonstrated it in these materials from other areas.

We have no information at this time to explain the irregular distribution of the organism in Great Lakes waters. Further studies are in progress to see if we can learn why Green Bay, for example, has a higher concentration of the organism than other places thus far examined.

It has been suggested that C. botulinum type E occurs only in the Northern Hemisphere above the 40th parallel. This idea was based largely on the fact that outbreaks of the disease have been limited to northern areas and that the organism has been found in nature only where the disease has occurred. But it must be remembered that until recently serious efforts to find the organism have been made only in response to outbreaks of type E botulism. The limited distribution of the disease might be associated with the food habits of the people rather than to the localized occurrence of the organism in nature.

A world wide survey for type E with emphasis on the Southern Hemisphere is being planned, but in the meantime, it can be stated that the organism has been found well below the 40th parallel. Johanneen (1936b) has isolated type E from mud collected in the harbor at Port Arthur, Texas, and, more recently, Ward and Carroll (1965) have found it in mud from Galveston Bay.

It is difficult at this time to assess the significance of C. botulinum type E to the meat industry. There is no reason to believe that the organism occurs on meats, but neither is there assurance that it does not. Our best consolation at the moment is that meat typically carries very few spore forming anaerobes of any type, and the chance of type E being present in significant numbers seems slim indeed.

On the basis of current knowledge it would appear that the best opportunity for contamination of meat with type E spores would exist in a retail market, where cross contamination could occur between fish and meat. The likelihood that this might happen coupled with circumstances that would allow growth and toxin production in a meat product that is not cooked before eating is impossible to evaluate. The prospect seems highly remote, however.
### Table 1. Geographic distribution of verified type E botulism outbreaks

<table>
<thead>
<tr>
<th>Country</th>
<th>Area</th>
<th>Outbreaks</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Hokkaido</td>
<td>29</td>
<td>222</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Northern Honshu</td>
<td>20</td>
<td>82</td>
<td>37</td>
</tr>
<tr>
<td>U. S.</td>
<td>Alaska</td>
<td>7</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Other states</td>
<td>8</td>
<td>37</td>
<td>15</td>
</tr>
<tr>
<td>Canada</td>
<td>British Columbia</td>
<td>8</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Labrador</td>
<td>3</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Sweden</td>
<td></td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Denmark</td>
<td></td>
<td>3</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Norway</td>
<td></td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>U.S.S.R.</td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 2. Outbreaks of type E botulism in the United States

<table>
<thead>
<tr>
<th>Year</th>
<th>State</th>
<th>Food</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1932</td>
<td>New York</td>
<td>Smoked salmon</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1934</td>
<td>New York</td>
<td>Canned sprats</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1941</td>
<td>California</td>
<td>Canned mushrooms</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1950-60</td>
<td>Alaska</td>
<td>Whale meat (5 outbreaks)</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmon eggs (2 outbreaks)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1960</td>
<td>Minnesota</td>
<td>Smoked ciscoes</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1961</td>
<td>Washington</td>
<td>Salmon eggs</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1963</td>
<td>Michigan</td>
<td>Canned tuna</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smoked whitefish</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Tennessee-Kentucky-Alabama</td>
<td>Smoked whitefish chubs</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>58</td>
<td>23</td>
</tr>
</tbody>
</table>
Table 3. Occurrence of \textit{C. botulinum} type E in the intestinal tract of fish from the Great Lakes

<table>
<thead>
<tr>
<th>Lake</th>
<th>No. fish tested</th>
<th>No. with type E</th>
<th>% with type E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erie</td>
<td>437</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>Superior</td>
<td>617</td>
<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>Huron</td>
<td>464</td>
<td>14</td>
<td>3.0</td>
</tr>
<tr>
<td>Michigan -- main body</td>
<td>1009</td>
<td>99</td>
<td>10.0</td>
</tr>
<tr>
<td>-- Green Bay</td>
<td>709</td>
<td>426</td>
<td>60.0</td>
</tr>
</tbody>
</table>

REFERENCES


12.


Johannsen, A. 1963b. Personal communication.


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DR. SULZBACHER: You heard about something which isn't a great problem in meat at the moment. It is something for people to think about, but there is one form of food poisoning that certainly is a serious problem. In the United States staphylococci are probably the most prevalent source of food poisoning and certainly are the source most usually associated with meats. I remember that way back in the middle thirties when I first started to become interested in this work, I worked for a man who got a great many samples of food that were suspected of having caused food poisoning and I used to get a lot of ham samples from him. I recall isolating numbers of Staphylococcus aureus from these ham samples and going back and saying there isn't anything in those hams that will
make anybody sick, there's nothing there but staph. That was, in fact, what I had learned in school (and in those days I was fresh out of school) and I thought I knew all about it. Well, I began to read a little bit and pretty soon I was telling my employer that we better give this a second thought; that the people at the University of Chicago are showing that Staphylococcus aureus was indeed a source of food poisoning. Well, that was all thirty some years ago and today we are all a lot wiser and a lot sadder and it certainly is, from the standpoint of the American meat industry, our most serious food poisoning problem. Dr. Dorothy Strong of the University of Wisconsin has dealt with this problem for a long time. She reminded me, when I asked her if she would be on this program, that in recent years she has been interested in another organism, Clostridium perfringens, which is also one of great concern to the meat industry, or should be, but we must leave that for another year. We'll get you well frightened this year and come back and hit you with perfringens another time. Because of Dr. Strong's experience with staphylococcus and the studies which she has made on staphylococcal poisoning from foods, I prevailed upon her to look back at her older work and tell us about it this morning....Dr. Strong.