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Sensitivity of an Enrichment Culture Procedure for Detection of *Clostridium botulinum* Type E in Raw and Smoked Whitefish Chubs

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The sensitivity of an enrichment culture procedure for detecting *Clostridium botulinum* type E in whitefish chubs (*Leucichthys* sp.) was assayed. Data demonstrated that fish inoculated with 10 or more viable *C. botulinum* spores regularly develop specifically neutralizable enrichment cultures. Mild heat treatment (60 C, 15 min) substantially reduced the sensitivity of enrichment culturing. This effect was particularly noticeable in the culturing of fish which harbored fewer than 10 spores each. Evidence is presented which indicates that sensitivity of enrichment, without heat, approaches the level of one spore per fish. Smoked whitefish chubs, containing from one to several hundred spores each, were examined for toxin content after storage at 5, 10, 15, and 28 C for as long as 32 days. The lowest temperature at which detectable toxin was produced was 15 C. This occurred in 1 of 10 fish incubated for 14 days. *C. botulinum* was regularly recovered, by enrichment culture, from fish inoculated with small numbers of spores, even though toxin was not detected by direct extraction of incubated fish. Persistence of *C. botulinum* type E spores was observed to decline with an increase in the temperature and time at which inoculated fish were stored.

Data previously reported from this laboratory (6, 7; P. J. Pace, E. R. Krumbiegel, R. Angelotti, and H. J. Wisniewski, Bacteriol. Proc., p. 10, 1966) clearly demonstrate the presence of *Clostridium botulinum* types in whitefish chubs collected from smoked-fish processors of the Milwaukee area. Although the methodology for the detection of *C. botulinum* was arrived at more or less empirically, various enrichment procedures were employed and an attempt was made to evaluate them qualitatively. It was concluded that (i) culturing the entire mass derived from ground fish is preferable to selecting a presumably representative 10-g portion of the same material, and (ii) employment of duplicate cultures, prepared from approximately equal amounts of ground material, one exposed to an internal temperature of 60 C for 15 min (heat shocked; HS) prior to being incubated and the other incubated without heat exposure (non-heat shocked; NHS), yielded more positive *C. botulinum* cultures than either method alone.

The prevalence of *C. botulinum* in whitefish chubs was found to be greatest at the processing stages prior to smoking. The organism occurred in approximately 20% of 102 samples collected from a stage prior to smoking (brine tank sample); however, only 10 of 858 (1%) smoked fish samples were found to harbor *C. botulinum*.

In reviewing these prevalence data, one might ask whether reported values represent minimal or actual contamination rates. An experimental format was designed to provide the following information: (i) the comparative efficiency of NHS versus HS in the development of toxin-containing enrichment cultures from fish harboring known numbers of *C. botulinum* type E spores; (ii) the minimal number of type E spores in or on fish which may be detected by the methods employed; (iii) the comparative efficiency of enrichment culturing as opposed to toxin extraction in the detection of *C. botulinum* in stored whitefish chubs.

MATERIALS AND METHODS

Preparation of spore suspensions, method I. A *C. botulinum* type E isolate, obtained from smoked whitefish chubs in this laboratory (6, 7), was used to produce spore crops. This isolate, designated S-32, 7 HS, was maintained in cooked liver medium (CLM) prepared

in accordance with a published formula (1). The stock culture, stored at 4 to 6 C, was reactivated by passage through three changes of CLM. These successive subcultures were incubated at 28 C for 48 hr. A few drops of the final CLM subculture were transferred into the depths of a Trypticase-peptone-glucose (TPG) broth (8). After 4 days of incubation at 28 C, 1.0-ml amounts of this TPG culture were transferred into plastic centrifuge bottles (250-ml capacity) containing 100 ml of TPG. These cultures were also incubated for 4 days at 28 C. Spore crop cultures were centrifuged at 900 $\times$ *g* for 1 hr at room temperature. Sedimented cells were taken up in 10 ml of gel-phosphate buffer, *p*H 6.2 (5). An equal volume of absolute ethyl alcohol was added to this suspension. After reaction at room temperature for 1 hr, with occasional shaking, the mixture was centrifuged for 1 hr as above. These sedimented spores were taken up in 20 ml of gel-phosphate buffer, washed twice as above, and stored at 5 to 7 C.

Preparation of spore suspensions, method II. A second spore suspension was prepared from isolate S-32, 7 HS, activated as above. However, this spore crop was produced in 100-ml volumes of TPG which were incubated for 40 hr at 28 C (as recommended by C. F. Schmidt, Continental Can Co., Chicago, Ill.). Incubation was terminated by immersing the centrifuge bottles in an ice bath. The suspensions were centrifuged as above and were resuspended in about 100 ml of cold, sterile, distilled water. This procedure was repeated for a total of three washings. Washed, sedimented, spore crops from four 100-ml cultures were then combined in 100 ml of distilled water and stored at 5 to 7 C.

Stock spore preparations were freed of macroscopic clumping by passage through sterile, absorbent cotton filters (10). Glass beads were incorporated into the stock suspension container and into containers used for subsequent serial dilutions, as a further aid for the dispersion of spores upon shaking.

Population counts of viable spores were prepared from dilutions in phosphate buffer (*p*H 7.0). Andersen's pork infusion-pea infusion medium (2) was used as a recovery substrate. Plates were enclosed in phosphorus ignition jars (9). Colony counts were made after 48 hr ($\pm$ 6 hr) of incubation at 28 C. Persistence of toxigenic *C. botulinum* type E was unfailingly corroborated by selection of isolates to CLM, followed by neutralization tests as previously described (6).

Assay of enrichment culture sensitivity. The stock spore suspension prepared by method I (ethyl alcohol-treated) was employed to introduce an inoculum ranging from approximately 100,000 spores per fish to fewer than 10 spores per fish. Dilutions were assayed by anaerobic plate count, on each day inoculation of fish took place, to determine the inoculum level. Fish employed in these experiments were obtained from two commercial fisheries operating in the Milwaukee area. The fish were selected from a processing stage at which a considerable bacterial population could be expected (brined and washed) and from one at which low contamination could be expected (fresh smoked; 7). These fish were exposed to spore dilutions by surface inoculation of 1.0-ml amounts in one set of experiments and by injection of 0.25 ml in each loin muscle in another set of experiments. Individual inoculated fish were then immediately processed for enrichment culturing as detailed previously (6). After incubation at 28 C for 7 days, cultures were examined for toxicity, neutralization, and *C. botulinum* isolates as previously described (6).

Enrichment culture sensitivity data were extended by use of low levels of spore inocula. A suspension prepared by method II was chosen for these studies. The experimental procedure for each day was similar to that described in the preliminary assay. However, only smoked fish were employed as the substrate. Ten experimental and 10 uninoculated controls were used each day. Inoculation was by surface exposure only. A working dilution of spores, in distilled water with glass beads, was prepared from the stock suspension for each of three experiments. Two of these experiments were conducted for 5 consecutive days each and the third for 10 days. The working dilution of spores was maintained at 5 C between daily inoculations. Ten replicate plates, containing 1.0 ml each of the inoculation spore suspension, were prepared each day. These plates were incubated anaerobically for 48 hr as previously described.

Inoculated pack studies. Surface inoculation of smoked fish was performed on three consecutive days with a spore suspension prepared by method I. On each day, fresh dilutions were prepared in phosphate buffer (*p*H 7.0). Tenfold serial dilutions of 1:100 through 1:1,000,000 were plated in duplicate on each day of inoculation. A selected dilution, 1:10,000 on day 1, 1:100,000 on day 2, and 1:1,000,000 on day 3, was used to surface-inoculate 45 fish with 1.0 ml each of spore suspension. Each fish was kept in a low-density, polyethylene, open-end, retail distribution bag during the inoculation procedure and subsequent storage. Three groups of 15 bags of fish were placed into three laboratory incubators. One group was kept at 5 C, one at 10 C, and one at 15 C. Fifteen packages of uninoculated fish (14 on one occasion) were included with each experimental run. These packages were stored in the 15 C incubator. Five packages of inoculated fish were removed from each storage chamber, along with five packages of uninoculated fish from the 15 C incubator, on days 8, 16, and 32 following the day of inoculation.

Assay for toxin and the presence of *C. botulinum* was performed on each individual fish. The fish were passed through sterile food choppers. Ground flesh was collected into appropriately identified, sterile beakers. Approximately 20 g of ground material was combined with 20 ml of cold gel-phosphate buffer. This composite was further comminuted in a Waring Blendor at low speed for 2 min. The resulting slurries were centrifuged at 12,000 $\times$ *g* in a Sorvall RC-2 centrifuge at 1.0 C for 30 min. Supernatant fluids were harvested and stored no longer than 2 hr at 5 C before being tested in mice. Each supernatant fluid was divided into three portions; one was adjusted to *p*H 5.5 to 6.5, if necessary. This portion was trypsin-activated and diluted prior to being inoculated into paired mice. Untreated and heated counterparts were also diluted and tested in mice as previously described (6).

Individual enrichment cultures were prepared from

the mass of ground flesh remaining after the removal of a 20-g portion for toxin extraction. Enrichment culture of these preparations was by NHS only because of the observed superior sensitivity as compared to HS enrichment. Incubation and toxin detection procedures were as previously described (6). At least one toxic culture from each group of five fish was also examined in complete neutralization tests (6).

Failure to detect toxin in these inoculated packs made it necessary to repeat the experiments at increased storage temperatures. The experimental approach remained essentially the same. A spore suspension, prepared by method II, was diluted to 10^{-4} in sterile distilled water. Forty smoked whitefish chubs (approximately 100 g each) were inoculated on each of 4 days. One half of the fish were inoculated with 1.0 ml by surface exposure. The other 20 fish were injected intramuscularly in each loin muscle with 0.25 ml of the same suspension. The fish were kept in open-end retail distribution bags and were stored in laboratory incubators. The 40 fish inoculated on days 1, 2, 3, and 4 were stored at 5, 10, 15, and 28 C, respectively.

TABLE 1. *Production of toxic enrichment cultures from fish injected intramuscularly with 0.5 ml each of various dilutions of Clostridium botulinum type E spore suspensions*

Sample point	No. of viable cells per inoculum	No. of toxic enrichment cultures/no. of fish cultured			
		Experimental[a]		Uninoculated Controls	
		NHS	HS	NHS	HS
(a) No. 6[b]	8×10^4	5/5	5/5	3/5[c]	0/5
	1.3×10^4	5/5	5/5	2/5[d]	0/5
	2.5×10^1	5/5	5/5	0/5	0/5
	6×10^1	5/5	2/5	0/5	0/5
	5.0	5/5	5/5	3/5[c]	1/5[d]
	0.14[e]	1/5	1/5	0/5	0/5
(b) No. 7[f]	3.2×10^4	5/5	5/5	0/5	0/5
	4.5×10^4	5/5	5/5	0/5	0/5
	9.8×10^2	5/5	5/5	0/5	0/5
	5×10^1	5/5	5/5	0/5	0/5
	4.0	5/5	1/5	0/5	0/5
	0.3[e]	1/5	0/5	0/5	0/5

[a] At least one culture of each group of five was neutralized and *C. botulinum* was recovered. All toxic supernatant fluids produced deaths typical of botulism.

[b] Brined and washed. Fish were approximately 100 g each.

[c] Nonspecific toxicity, late deaths.

[d] One toxic culture was neutralized and *C. botulinum* was recovered; the balance was nonspecific.

[e] A viable cell was found in 14/100 and 3/10 inocula.

[f] Fresh smoked. Fish were approximately 100 g each.

Twenty replicate plates of 1.0-ml samples of the inoculating suspension were prepared in pork infusion-pea infusion agar each day. These plates were incubated in phosphorus ignition jars at 28 C as before. However, colony counts were recorded from 10 plates after 48 hr and from the other 10 plates after 168 hr.

Extracts were prepared from 10 fish on each assay day. Assay days were selected to represent 7, 14, 21, and 28 days of storage at each temperature (5, 10, 15, and 28 C). Five fish inoculated intramuscularly and five surface-inoculated fish were examined on a given day. Extracts were prepared as above, except that the entire ground mass from each fish was blended with 50 ml of gel-phosphate buffer. Supernatant fluids from the homogenates were harvested and tested for toxicity as above. It was noted that some of these supernatant fluids tended to solidify upon overnight storage at 5 C. The exudates recovered from gelled extracts were found to have lost toxicity. Therefore, subsequent screening tests were modified to include a pair of mice passively immunized with type E antitoxin. Complete neutralization tests (with monovalent antitoxin types A, B, C, D, and E) were also performed,

TABLE 2. *Production of toxic enrichment cultures from fish surface inoculated with 1.0 ml each of various dilutions of Clostridium botulinum type E spore suspensions*

Sample point	No. of viable cells per inoculum	No. of toxic enrichment cultures/no. of fish cultured			
		Experimental[a]		Uninoculated Controls	
		NHS	HS	NHS	HS
(a) No. 6[b]	6.7×10^3	5/5	5/5	1/5[c]	1/5[d]
	3.8×10^3	5/5	5/5	1/5[c]	0/5
	3.7×10^2	5/5	5/5	3/5[d]	0/5
	7.3×10^1	5/5	1/5	2/5[d]	0/5
	10^1	5/5	1/5	2/5[d]	0/5
	1.3[e]	3/5	3/5	0/5	0/5
(b) No. 7[f]	8×10^4	5/5	5/5	0/5	0/5
	1.9×10^4	5/5	5/5	0/5	0/5
	1.7×10^3	5/5	5/5	1/5[d]	0/5
	1.9×10^2	5/5	5/5	0/5	0/5
	3.3[e]	5/5	5/5	0/5	0/5
	1.1[e]	4/5	1/5	0/5	0/5

[a] At least one culture of each group of five was neutralized and *C. botulinum* was recovered. All toxic supernatant fluids produced deaths typical of botulism.

[b] Brined and washed. Fish were approximately 100 g each.

[c] Nonspecific toxicity, late deaths.

[d] One toxic culture was neutralized and *C. botulinum* was recovered, balance nonspecific.

[e] The numbers 1.3, 3.3, 1.1 represent 13, 33, and 11 cells distributed among ten 1.0-ml inocula.

[f] Fresh smoked. Fish were approximately 100 g each.

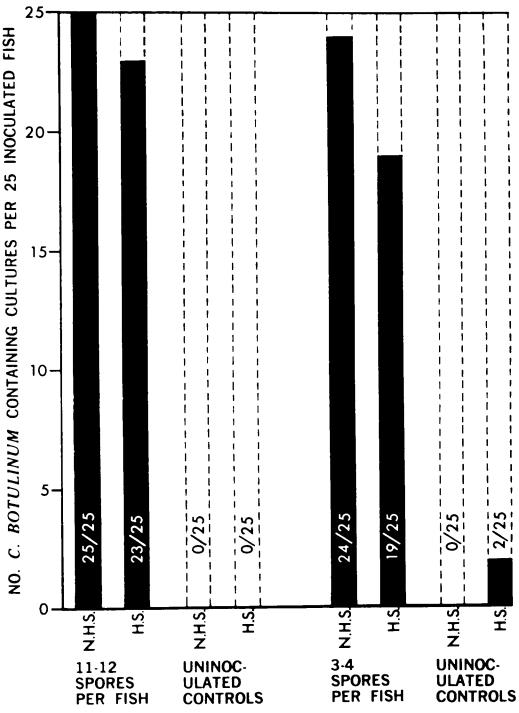

FIG. 1. *Comparison between nonheat shock (NHS) and heat shock (HS; 60 C for 15 min) on development of toxic enrichment cultures. Smoked whitefish chubs were surface-inoculated with low numbers of Clostridium botulinum type E spores.*

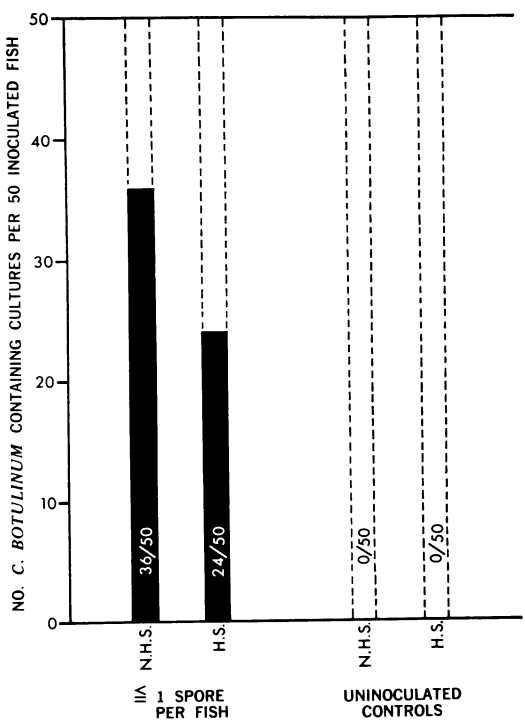

FIG. 2. *Development of toxic enrichment cultures from smoked whitefish chubs each surface-inoculated with approximately one Clostridium botulinum type E spore. Nonheat shock (NHS) versus heat shock (HS; 60 C for 15 min).*

when indicated, with stored extracts which had not gelled overnight.

RESULTS

Assay of enrichment culture sensitivity. It is clear, from Tables 1a, 1b, 2a, and 2b, that fish need harbor only very small numbers of viable *C. botulinum* type E spores in order to develop toxic enrichment cultures. NHS enrichment always resulted in the development of toxic cultures when the inoculated fish contained three or more viable spores. Two entries of Table 1 indicate that, of the 1-ml inocula used, 1.4 of 10 and 3 of 10, respectively, contained a viable spore. Each of these diluted suspensions resulted in one out of five fish developing toxic enrichment cultures by NHS. Furthermore, each culture yielded isolates of type E *C. botulinum*. A recovery rate of one of five correlated with calculated inocula levels. These data suggest that one viable spore per fish may be detected with some consistency by NHS enrichment. Examination of Table 2 reveals that, of 20 fish surface-inoculated with approximately 1 spore each, 7 of 10 produced toxic cultures by NHS and 4 of 10 by HS. Therefore, mild heat shocking (60 C, 15 min) appeared to affect adversely the development of toxic enrichment cultures from fish harboring small numbers of type E spores. This deleterious effect is shown in Fig. 1 and 2.

Table 3 shows the distribution of viable spores among 100 replicate platings of 1.0 ml of the inoculum suspension employed in the experiment illustrated in Fig. 2. It can be seen that none of 30 replicate, 1.0-ml platings, prepared on days 1, 4, and 5, yielded a viable spore after 48 hr at 28 C. However, 13 of 15 fish inoculated on those days produced toxic enrichment cultures by NHS and 5 of 15 by HS after 7 days at 28 C. Whether this observation resulted from chance selection or whether it reflects that spores grow out more readily in enrichment culture cannot be determined with these data. The enrichment cultures were incubated for 7 days, as opposed to 2 days for the plates. Therefore, the discrepancy may reflect nothing more than an extended lag phase. It is not likely that the toxic experimental cultures resulted from natural contamination of the fish. As can be noted from Fig. 1 and 2, 200 uninocu-

lated smoked fish were included as controls. Only 2 (1%) of these were found to contain *C. botulinum*.

Inoculated pack studies. These studies were not designed to determine the effect of the salt content of experimental fish upon spore outgrowth. However, 30 smoked fish, collected at the same time as those in the experiments listed in Table 4, were analyzed for NaCl in the water phase of loin muscles by a prescribed method (3). The salt content was found to range from 1.2 to 3.4%, with a mean of 2.4%. None of the inoculated fish having salt contents approximating these levels contained toxin after prolonged storage at 5, 10, or 15 C. Persistence of type E spores was regularly detected in fish stored as long as 32 days at 5 C. As a matter of fact, Table 4 reveals an enrichment culture sensitivity approaching one spore per fish at this storage temperature. Table 4 indicates that prolonged storage of fish having small numbers of type E spores at 10 and 15 C materially reduced the detection of *C. botulinum* by enrichment culturing.

TABLE 3. *Production of toxic enrichment cultures from smoked whitefish chubs surface: inoculated with a single viable spore of Clostridium botulinum type E*

Day	Distribution of viable spores in each of 10 inocula (1.0 ml)[a]	No. of fish producing toxic enrichment cultures/no. inoculated	
		NHS	HS
1	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	4/5	2/5
2	1, 1, 0, 0, 0, 0, 0, 0, 0, 0	4/5	3/5
3	1, 0, 0, 0, 0, 0, 0, 0, 0, 0	2/5	4/5
4	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	5/5	1/5
5	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	4/5	2/5
6	1, 0, 0, 0, 0, 0, 0, 0, 0, 0	4/5	2/5
7	1, 1, 1, 1, 1, 0, 0, 0, 0, 0	3/5	2/5
8	1, 1, 2, 0, 0, 0, 0, 0, 0, 0	4/5	4/5
9	4, 1, 1, 1, 1, 0, 0, 0, 0, 0	1/5	3/5
10	1, 1, 1, 0, 0, 0, 0, 0, 0, 0	5/5	2/5

[a] Pork infusion-pea infusion agar, 48 hr at 28 C in phosphorus ingition jar (NHS).

The assay level used for the detection of toxin in these fish was calculated to be 40 mouse MLD per gram. This figure was derived from the 1:2 dilution used for blending, which was further diluted 1:10 before being injected into mice in 0.5-ml amounts. Therefore, it is not known whether low levels of toxin were produced in the stored fish. Similar calculation has set the minimal toxin detection level at 20 MLD per ml of enrichment culture supernatant fluid.

An experiment was designed to determine whether inocula containing larger numbers of spores might produce toxin in smoked fish at the same temperatures. An additional storage temperature of 28 C was also included in these studies (Table 5). Each experimental fish weighed at least 100 g. Homogenates were prepared with 50 ml of

TABLE 4. *Effect of time and temperature of storage on toxin development and spore survival in surface-inoculated smoked whitefish chubs*[a]

No. of spores	Storage (days)	Storage temp								
		5 C			10 C			15 C[d]		
		Extract[b]	Enrichment cultures[b]	Type[c]	Extract	Enrichment culture	Type	Extract	Enrichment culture	Type
(a) 14[e]	8	0/5	5/5	1/1 E	0/5	5/5	1/1 E	0/5	5/5	1/1 E
	16	0/5	5/5	1/1 E	0/5	5/5	1/1 E	0/5	4/5	1/1 E
	32	0/5	5/5	1/1 E	0/5	5/5	1/1 E	0/5	3/5	1/1 E
(b) 3.5[e]	8	0/5	5/5	1/1 E	0/5	4/5	1/1 E	0/5	5/5	1/1 E
	16	0/5	5/5	1/1 E	0/5	5/5	1/1 E	0/5	4/5	1/1 E
	32	0/5	5/5	1/1 E	0/5	4/5	1/1 E	0/5	1/5	0/1
(c) 0.5[e]	8	0/5	3/5	1/1 E	0/5	2/5	1/1 E	0/5	1/5	1/1 E
	16	0/5	3/5	1/1 E	0/5	0/5		0/5	0/5	
	32	0/5	3/5	1/1 E	0/5	1/5	1/1 E	0/5	0/5	

[a] Approximately 100 g each.
[b] Number toxic per number tested.
[c] Number neutralized per number tested and toxin type.
[d] An uninoculated control, also stored at 15 C, produced consistently negative results.
[e] Viable type E *Clostridium botulinum* spores per inoculum (1.0 ml).

TABLE 5. *Toxin development in smoked whitefish chubs inoculated with type E Clostridium botulinum spores*

Storage time (days)	Route of inoculation[a]	No. of fish containing toxin/no. examined after storage at			
		5 C	10 C	15 C	28 C
7	Surface	0/5	0/5	0/5	1/5[b]
	Intramuscular	0/5	0/5	0/5	4/5[b]
14	Surface	0/5	0/5	1/5[c]	1/5[c]
	Intramuscular	0/5	0/5	0/5	3/5[c]
21	Surface	0/5	0/5	0/5	2/5[c]
	Intramuscular	0/5	0/5	0/5	1/5[c]
28	Surface	0/5	0/5	0/5	0/5
	Intramuscular	0/5	0/5	0/5	0/5
No. of viable spores per ml of inocula					
48 hr of incubation		638	373	398	389
168 hr of incubation		457	1,140	671	889

[a] Surface inoculation, 1.0 ml; intramuscular inoculation, 0.5 ml.

[b] Extract gelled when stored overnight at 5 C; toxicity lost.

[c] Type E toxin was confirmed by specific neutralization.

gel-phosphate buffer. Therefore, the minimal toxin assay level was calculated to be about 30 mouse MLD per gram of fish. Toxin was detected in 1 of 40 inoculated fish stored at 15 C, whereas 12 of 40 inoculated fish stored at 28 C were observed to contain toxin.

Viable-spore counts were determined from the average of ten 1.0-ml replicate plates prepared in duplicate each day. In three of four determinations, viable-spore counts determined after 7 days at 28 C were 41 to 67% higher than those made after 48 hr of incubation. Thus, it appears that the discrepancy noted in the experiments shown in Fig. 2 may in fact be due to an extended lag phase of the spores employed in the inocula.

DISCUSSION

Little information is available concerning the number of spores necessary for the detection of *C. botulinum* in environmental specimens. However, Dolman (4) cited K. F. Meyer as estimating that 1,000 to 10,000 spores per gram of soil are necessary for toxin detecton. Data contained in this communication reveal that an enrichment culture procedure, as applied in an earlier surveillance study (6, 7; P. J. Pace, E. R. Krumbiegel, R. Angelotti, H. J. Wisniewski, Bacteriol. Proc., p. 10, 1966), has a detection sensitivity approaching 1 spore per fish (approximately 100 g). Mild heat treatment (60 C, 15 min) prior to incubation of enrichment cultures reduces the sensitivity of the method when one examines fish harboring fewer than 10 viable spores each. The use of mild heat treatment, however, virtually eliminated the problem of nonspecific deaths (cf. Tables 1 and 2). Such deaths occurred only in mice inoculated with supernatant fluids of NHS cultures. A similar observation was made during surveillance studies (6, 7; P. J. Pace, E. R. Krumbiegel, R. Angelotti, H. J. Wisniewski, Bacteriol. Proc., p. 10, 1966), in which NHS cultures produced more nonspecific deaths than their HS counterparts.

It is interesting to compare Fig. 2 and Table 3 with data reported by Schmidt et al. (8). In an examination of four type E strains, these investigators observed viable spore counts to range from 40 to 68% of the refractile spores present in the suspensions. These viable spore assays were also made without the use of heat treatment. From Fig. 2, it is seen that 36 of 50 fish, each inoculated with 1.0 ml of spore suspension, produced toxic cultures by NHS enrichment. The outgrowth of 24 viable spores resulted from 100 replicate plates, each containing 1.0 ml of the suspension. Simple calculation ($36/50 \times 2/2 = 72/100$) indicates a plate count viability of 33% ($24/72 = 0.33$) of the toxic enrichment cultures produced. One might speculate that these data indicate that fish homogenates in TPG provide a more suitable environment for spore outgrowth than does Andersen's pork infusion-pea infusion agar. Schmidt (8) suggests that the short incubation time (40 hr in his study and 48 hr in the present study) may cause the apparent reduced viability of spores assayed by colony outgrowth as opposed to refractile counts. This proposal is supported by the data in Table 5. Three of four suspensions subjected to replicate platings were found to yield 41 to 67% higher counts when allowed to incubate for 168 hr (7 days) as opposed to 48 hr.

The enrichment culture procedure evaluated herein has been found to be highly sensitive for the detection of *C. botulinum* type E spores in or on both brined and smoked whitefish chubs. Consequently, it should find useful application not only in surveillance studies but also in assaying the efficiency of various processing methods proposed to render whitefish chubs free from type E *C. botulinum*.

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