Low-Temperature Irradiation of Beef and Methods for Evaluation of a Radappertization Process

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An inoculated, irradiated beef pack (1,240 cans) study was conducted for the determination of microbiological safety for unrestricted human consumption. Each can contained a mixture of $10^9$ spores of each of 10 strains of Clostridium botulinum (5 type A and 5 type B), or a total of $10^7$ spores/can. The cans were irradiated to various doses (100 cans/dose) with $^{60}$Co gamma rays at $-30 \pm 10$ C, incubated at $30 \pm 2$ C for 6 months, and examined for swelling, toxicity, and recoverable botulinal cells. The minimal experimental sterilizing dose based on nonswollen, nontoxic sterile cans was $2.2 < \text{experimental sterilizing dose} \leq 2.6$ Mrad. Using recoverable cells as the most stringent criterion of spoilage, and assuming the conventional simple exponential (without an initial shoulder) rate of spore kill, the "12D" dose was 3.7 Mrad when estimated on the basis of a mixture of 10 strains totaling $10^9$ spores/can, and 4.3 Mrad if it is assumed that each can of beef contained $10^6$ spores of a single most resistant strain and all of these spores were of identical resistances. However, an analysis of the data by extreme value statistics indicated with 90% confidence that the spore death rate was not a simple exponential but might be a shifted exponential (with an initial shoulder), Weibull, lognormal, or normal, with a "12D" equivalent of about 3.0 Mrad regardless of the initial spore density per can. There was an apparent antagonism between the irradiated type A and B strains in the cans. Some of the cans contained type B toxin but did not include type B viable cells. Other cans had a mixture of type A and B toxins, but a large number of these cans did not yield recoverable type B cells. However, type A viable cells could always be demonstrated in those cans containing type A toxin.

Some food products can withstand the effects of sterilizing doses of ionizing energy better than other foods. Prototype radappertization (24) processes for several of the less sensitive items such as bacon, ham, pork sausage, corned beef, pork, and codfish cake have already been reported (3, 4, 6, 7).

Under conventional handling conditions, beef is one of the most radiation-sensitive foods; it experiences undesirable changes in organoleptic qualities even with low levels of radiation. But recent technological developments in the U.S. Army Natick Development Center (NDC) have produced organoleptically acceptable beef after exposure at $-30 \pm 10$ C to radiation doses up to 7.1 Mrad (E. Wierbicki, unpublished data). Hence, NDC has initiated a comprehensive long-range investigation of the wholesomeness of beef radappertized at $-30 \pm 10$ C with $^{60}$Co gamma rays and with 10 MV electrons to determine its safety for unrestricted human consumption (43, 45). Currently, an inoculated pack study was conducted to determine the minimal radiation dose (MRD) necessary to attain microbiological safety for consumption of radappertized beef.

Many reports are available on the microbiological aspects concerning the irradiation of beef products. Using uninoculated beef, Bushkanets and Ladukhina (13) obtained sterility with 3.0, but not with 2.0, Mrad and Hannan (32) with 2.79 but not with 1.86 Mrad; they did not indicate the radiation temperature used. Cipolla and Denti (17) sterilized the meat with 2.0 Mrad at 25 C and Coleby et al. (18) reported that 5 Mrad produced sterility at a radiation temperature of $-75$ C. Cain et al. (14) could not quite sterilize uninoculated ground beef (contaminated with an indigenous microflora of 8.3 X $10^6$g) with 1.96 Mrad irradiated at 21.1 C either with a single exposure (13 survivors) or with the dose fractionated up to eight equal increments 4 h apart (two survivors). They also found that the microbial resistance decreased
in the beef as the radiation temperature (−17.8 to 65.6 °C) increased, until at 65.6 °C the temperature alone was lethal.

Much more information is available on inoculated beef products. Some of the results were based upon dose-survival curves, whereas other data were collected from partial spoilage (quantal) responses. Erdman et al. (23) inoculated chopped beef with a type A strain of Clostridium botulinum spores ranging from 10^6 to 10^9/g and found that 1.86 Mrad delivered at room temperature reduced the spore density to 0.08%; five type E strains tested were significantly more sensitive than the type A strain. Wheaton et al. (52) inoculated cans of ground beef with individual cultures of type A (three strains) and type B (two strains) of botulin spores, froze the cans to −29 C, irradiated them in a frozen state (final temperature unknown) to 0.85 or 1.7 Mrad, and estimated the number of surviving spores. An initial population of 5.9 × 10^9/g of meat of the most resistant strain, 12885A, was reduced to 2.1 × 10^6/g (36%) by 0.85 Mrad and to 1.9 × 10^5/g (0.3%) by 1.7 Mrad. The latter dose was also insufficient to sterilize their uninoculated controls. El-Bisi et al. (21) inoculated single thin slices of beef contained in individual test tubes with 10^9 spores of C. botulinum strain 53B, irradiated them at various controlled temperatures (−196 to 20 °C), and obtained D values (90% spore reduction doses on the linear portions of the semilog survival curves). As did Cain et al. (14) for indigenous organisms, they found that the D values increased with decreases in radiation temperature. Although El-Bisi et al. (21) did not irradiate at −30 C, the temperature of greatest concern to us, the “12D” dose, which has been recommended (35, 36, 47) as the MRD for microbiological safety, was 3.76 Mrad at −20 C and 3.96 Mrad at −40 C; when they included the shoulder (L) in their estimate (as “12D” + L), their MRDs increased to 4.13 and 4.35 Mrad, respectively.

Grecz and co-workers (25–28) confirmed the influence of decreasing radiation temperatures on the increased resistance of C. botulinum spores with inoculated beef pack partial spoilage data. Using precooked ground beef seeded with strain 33A spores (2.3 × 10^6/can) and irradiating at temperatures in the range −195 to 95 °C, they found a linear increase in resistance with a decrease in temperature (28); the MRD at −30 C computed to 4.93 Mrad. Employing cooked ground beef containing 5 × 10^6 spores of botulinal strain 213B per can, Kempe et al. (38) obtained an experimental sterilizing dose between 3.18 and 3.43 Mrad at a radiation temperature near 4 C; with raw ground beef the experimental sterilizing dose was between 2.56 and 2.79 Mrad (39), indicating that a change in the treatment of a food substrate can alter significantly the radiation dose requirements. Anderson et al. (2) irradiated ground round beef containing botulinal spores (10^9/can) up to 3.0 Mrad at 20 C; the most resistant strain of three tested, 33A, spoiled 15 of 25 cans at this dose, indicating a “12D” value of 5.3 to 5.4 Mrad when estimated by the conventional method (9, equations 1 and 3). Schmidt and his colleagues conducted a series of inoculated pack studies with C. botulinum spores. They compared the radiation resistances of types A (six strains), B (five strains), and E (six strains) in beef stew at ambient temperature and found that the D values of types A and B overlapped; based upon the average D's, the order of resistances were A > B > E (0.279, 0.238, and 0.132, respectively) (49), verifying the observations of Erdman et al. (23) that type E spores were more sensitive in beef than type A spores. The most resistant of the 17 strains used by Schmidt et al. (49) was 109A. Employing a mixture of three type A, which included 109A, and two type B strains (2 × 10^7 spores/stain) in cooked beef steak, they reported a mean D value of 0.31, or a radappertition dose of 3.73 Mrad (48).

Obviously, the variable results obtained in the above studies were due to the many differences in the experimental conditions used. From these investigations it appears that an MRD approaching 4 to 5 Mrad would be required to make beef products microbiologically safe if C. botulinum were present in very high numbers.

A single outbreak of botulism (type B) attributed to commercially canned potted beef occurred in 1925 in the United States. Since that incident no further outbreaks ascribed to commercially canned beef products occurred through October 1974 (41, 51). In November 1974 an outbreak (two cases, one death) of type A botulism occurred due to commercially canned beef stew (51). Five additional outbreaks implicating home canned beef were reported in the 75-year period 1899–1974 (41, 51).

In the last 16 years NDC examined a variety of uninoculated beef products (6, 115 samples), unirradiated and irradiated (0.1 to 7.1 Mrad), for C. botulinum. All samples were negative. Despite the history of a very low occurrence of botulinal spores in beef and other meat products (3, 30), the organism could be expected to be present in beef at least in small numbers. Hence a successful radiation sterilization process must be capable of destroying these spores.
in numbers very much higher than have been observed in the food.

This paper reports on a prototype radappertization process at \(-30 \pm 10\) C for beef undergoing intensive wholesomeness studies, using \(C.\ botulinum\) spores types A and B as the indicator of microbiological safety and \(^{60}\)Co gamma rays as the radiation source, and possible methods for evaluating an MRD.

**MATERIALS AND METHODS**

**Beef preparation.** Deboned and defatted beef chunks of commercial grade or better (USDA inspected) were vacuum mixed with 0.75% sodium chloride (noniodized), 0.38% sodium tripolyphosphate, and 3% chipped ice, stuffed tightly into 6.5 Visking casing (Union Carboide), tied off under pressure to remove entrapped air, and placed at \(-2\) to \(3\) C for not less than 1 h to permit the salts to equilibrate throughout the beef rolls. The meat was then exposed to a temperature of 50 to 60 C for 3 h, 66 to 71 C for 6 h, and 90 C until the center temperature of the rolls attained 68 to 75 C. This heat treatment inactivated the autolytic beef enzymes and formed a protective protein outer coat on the rolls, which prevented excessive loss of moisture from the meat. This process is identical to that used for beef currently undergoing laboratory animal feeding studies to determine the wholesomeness of the radappertized product for unlimited human consumption (43, 45). The product was then chilled to \(-3\) to \(5\) C, the casings were removed, and the rolls were diced in a slicer-dicer to 1.27-cm cubes, packed in 40 \(\times\) 5-g quantities in a 5 to 10 C room into epoxy enamel metal cans (211 by 101.5), loosely closed with lids, leaving a 0.6-cm headspace per can, and held at 1 to 3 C until inoculated 24 h later. Sanitary precautions were followed throughout the handling operation, including the prior autoclaving of the cans, lids, and other food-handling equipment for 15 min at 121 C.

Proximate chemical analysis (11) on uninoculated unradappertized random cans of beef was performed, using 10 samples, four cans per sample (about 160 g), and assayed in duplicate. Results are listed in Table 1.

**Test organism.** Five type A (33A, 36A, 62A, 77A, 12885A) and five type B (9B, 40B, 41B, 53B, 67B) strains of \(C.\ botulinum\) were used. These organisms, described previously (8), had been employed in other inoculated pack studies (3, 4, 6, 7), except that strain 67B was substituted for 51B. Spore crops of each strain were produced and harvested as cited earlier (5), using 2% \((\text{NH}_4)_2\text{SO}_4\) as the liquid phase, and stored at 1 to 3 C until needed.

**Enumeration of spores.** Spores were heat shocked at 80 C for 10 min (which inactivated vegetative cells and residual botulinum toxin), cooled rapidly in ice water, and diluted decimally in chilled, sterile distilled water. One-milliliter aliquots were inoculated into each of triplicate tubes (11 by 202 mm) containing 0.3 ml of filter-sterilized 5% \(\text{NaHCO}_3\), and molten agar medium was added to the tubes. Pouring of the agar into the tubes produced uniform mixing of the spores by the resulting swirling motion with the tube contents. The tubes were stratified with 1.5 to 2 cm of the same medium and incubated at \(30 \pm 2\) C for 30 to 40 h, and the numbers of colonies formed in the triplicate tubes were averaged. Each spore test sample was enumerated in duplicate; hence the average count represented a total of six count tubes per dilution.

The medium (TYT) contained 5% thionite (BBL), 0.5% yeast extract (BBL), 0.5% Tryptase (BBL), 0.05% sodium thioglycolate, and 0.75% plain agar (Difco), and the pH was adjusted to 7.2 with 5 N \(\text{NaOH}\) before autoclaving. This medium produced, generally, higher colony counts and less gassing than the unfortified thionite agar used previously (5).

**Inoculation and can sealing.** Heat-shocked and chilled (1 to 3 C) spores of each strain were mixed in equal numbers (10\(^{9}\)/strain) to yield a single inoculum of 10\(^{6}\) spores/ml, and the cans of beef were inoculated with 1.0-ml aliquots by automatic syringe (Filametic vial filler, model AB, National Instrument Co., Inc., Baltimore). The suspension, spread uniformly over the surfaces of the packed beef cubes, permeated through the crevices of the meat and the open spaces in the cans. The cans were vacuum sealed to 125 mm of Hg and stored at \(-40\) \(\pm\) 5 C until irradiated. Including appropriate controls, a total of 1,240 cans were involved in this study.

**Irradiation.** The cans were irradiated at \(-30 \pm 10\) C with \(^{60}\)Co gamma rays. The dose rate was 3.93 \(\times\) 10\(^4\) rad/min, and the transient dose was 1.95 \(\times\) 10\(^4\) rad. The temperature was controlled and monitored as described by Jarrett (34). The doses used were in the range 1.4 to 5.0 Mrad in increments of 0.4 Mrad. Lots of 100 cans were simultaneously exposed to each dose; in addition, the dose distribution within

| Value   | Protein (%) | Fat   | \(\text{H}_2\text{O}\) | \(\text{NaCl}\) | \(p^a\) | Ash  | \(pH\) |
|---------|-------------|-------|------------------------|-----------------|-------|------|-------|
| Range   | 20.98–22.69 | 12.69–16.27 | 59.61–61.93 | 0.53–0.63 | 0.275–0.287 | 1.96–2.10 | 6.1   |
| Median  | 22.24       | 13.92 | 61.14                  | 0.57            | 0.282  | 2.06 | 6.1   |

\(^a\) See reference 11. Duplicate determinations were made on 10 random uninoculated nonirradiated samples. Each sample consisted of the entire pooled contents of four replicate cans (160 \(\pm\) 20 g).

\(^b\) As sodium tripolyphosphate.
each lot of cans was also determined, so that the dose of every irradiated can was known.

Assay for spoilage. All irradiated cans and their controls were incubated at 30 ± 2 °C for 6 months. They were examined daily (except on weekends) for swelling for the first month and weekly thereafter. At the end of the incubation period the cans were stored at 1 to 3 °C until assayed. Analyses for C. botulinum toxin and survivors began at the highest dose yielding 100% swollen cans and included all swollen and flat cans at higher doses.

The can lids were sanitized by scrubbing with a brush in a solution of detergent, rinsing in tap water, and drying with clean tissues, and then they were sterilized by adding 95% ethanol and flaming. Swollen cans were individually enclosed within a sterile inverted funnel, a sterile (95% ethanol and flame) stainless-steel punch (0.3 by 30 cm) was inserted through the funnel stem, and a hole only large enough to permit the can gases to escape was made in the lids with a gentle hammer blow on the protruding end of the punch. No beef solids or juices ever escaped from inside the cans with this technique.

Employing aseptic technique, the cans were opened, and the entire contents of each can were transferred (with the aid of a disposable tongue depressor) to 1,000-mL Waring aluminum blender jars, diluted 1:5 (wt/vol) with gelatin-phosphate (0.2% gelatin + 0.4% Na2HPO4 and adjusted to pH 6.2 with HCl), and blended for 3 min at highest speed. Samples of homogenate (30 mL) were centrifuged (1,465 × g for 20 min), and one white male mouse (strain CD-1, 15 to 20 g) per sample was injected intraperitoneally with 0.5 mL of supernatant fluid. Every sample producing death or illness of the mouse within 4 days of injection was retested on two unprotected mice, two mice protected with botulinum antitoxin type A, two with antitoxin type B, two with a mixture of antitoxins types A and B, and two unprotected mice received 0.5 mL of supernatant which had been boiled for 10 min.

To detect the presence of viable botulinum cells, 10 mL of homogenate was inoculated into a 60-mL screw-capped bottle containing 40 mL of air-in-sterile TYT (without agar) supplemented with 0.5% glucose and 0.6 mL of 5% NaHCO3. To insure the recovery of surviving dormant spores an identical bottle of medium was similarly inoculated, heat shocked at 80 °C for 10 min, and cooled rapidly to about 30 °C. All inoculated bottles were incubated at 30 ± 2 °C for 30 days. The appearance of turbidity within this period was confirmed for C. botulinum with the mouse toxicity test described above.

As a precaution, a reserve 30-mL sample of all homogenates and supernatants was retained at 1 to 3 °C until all tests were completed.

Computation of a radiation process. The prototype radiation process was based upon the recommended (35, 36, 47) "12D," or MRD, concept of safety. Since there is still a question concerning the rate of spore death in an inoculated pack, our MRD calculations reflected simple exponential (without an initial shoulder), shifted exponential (with an initial shoulder), Weibull-, lognormal-, and normal-type spore kill; these are among the most common distributions observed in nature. Extreme value statistics (44; unpublished data) were used to compute all these rates of spore death; this form of statistics takes into account both the probability of spore kill in a can and the probability of can sterilization with changes in dose. In addition, the simple exponential and normal rates of spore death were estimated by the conventional techniques (9, equations 1 and 3; 10).

RESULTS

Experimental sterilizing dose. Lots of 100 cans of beef per dose containing a total of 9.8 × 10⁶ spores (9.8 × 10⁶ spores/can times 100 cans/dose) and irradiated to 2.6 Mrad or higher were nonswollen, nontoxic, and sterile (Table 2). Exactly one-half of a similar lot of 100 cans subjected to 2.2 Mrad was swollen, contained toxin, and had botulinum cells; an additional six cans were flat and nontoxic but harbored viable but dormant C. botulinum. All cans irradiated to 1.8 Mrad were swollen, toxic, and had recoverable botulinum cells. The experimental sterilizing dose, therefore, was 2.2 < experimental sterilizing dose ≤ 2.6 Mrad and the dose at which 50% of the cans are sterilized (LD50) was 2.224 Mrad.

Cans of beef exposed to less than 1.8 Mrad produced 100% visible spoilage (swelling) in only 3 days of incubation at 30 ± 2 °C, these samples were not deemed necessary for the examination of toxin and viable C. botulinum. Cans that received 1.8 Mrad became 100% swollen in just 4 days of incubation. In the same

| Radiation dose (Mrd) | No. of cans of beef | Swollen | With toxin | With viable C. botulinum |
|---------------------|---------------------|---------|------------|-------------------------|
|                     | Tested              | A       | B         | A + B                  |
|                     |                     | A       | B         | A + B                  |
| 0                   | 100                 | 100     | NT        | NT                     |
| 1.4                 | 100                 | 100     | NT        | NT                     |
| 1.8                 | 100                 | 100     | 0         | 100                    |
| 2.2                 | 100                 | 50      | 30        | 10                     |
| 2.6                 | 100                 | 0       | 0         | 0                      |
| 3.0 - 5.0³         | 600³                | 0       | 0         | 0                      |

a A mixture of 10 strains (5 type A and 5 type B), 10⁶ spores/strain or 9.8 × 10⁶ spores/can, was used.

b Average of each 100-can lot.

c NT, Not tested.

d Doses increase in 0.4-Mrad increments.

* 100 cans/dose.

Table 2. Effect of ⁶⁰Co irradiation at 30 ± 10°C on spoilage of beef inoculated with C. botulinum spores.
period of time, samples of meat subjected to 2.2 Mrad had 11 swollen cans out of the 50 (22%); in 15 days of incubation 45 (90%) of the cans swelled, but it required 64 days for visible swelling, and the number of toxic samples, to reach a maximum (50 cans) (Fig. 1a). The maximum rate of swelling occurred on day 4 of incubation; after the first week of incubation the swelling rate decreased considerably (Fig. 1b).

Minimal sterilizing dose. The most severe criterion of beef spoilage, botulinal survivors (Table 2), was used to estimate the "12D" dose. Since there was only one significant partial spoilage "point," 2.2 Mrad (Table 2), just the simple exponential and normal death rates could be computed by conventional methods (9, equations 1 and 3).

The MRD calculation based upon the alternative assumption that spore kill may follow one of a number of other distributions commonly found in nature (normal, lognormal, Weibull, or shifted exponential) requires several partial spoilage points. Since the dose of every can in each lot was known, the 100 replicate cans were divided into five sets of 20 cans each in ascending doses, and the individual doses within each set of cans were averaged. The results were tabulated both for the 1.8- and 2.2-Mrad lots (Table 3).

Using the five partial spoilage points at 2.2 Mrad (Table 3), the MRD values were estimated based upon the various assumed spore death rates which could occur in an inoculated pack (Table 4). Computations were made both on the basis of a single most resistant strain (10⁶ spores/can) and on the presence of a competitive mixture of 10 strains (10⁶ spores/can) in the beef. Employing the conventional simple exponential calculations (9, equations 1 and 3), the "12D" dose was 4.2 to 4.3 Mrad when we assumed that we had 10⁶ spores/can and 3.6 to 3.7 Mrad when we assumed that we had 10⁷ spores/can. Estimates for all the remainder of the above-assumed distributions gave 3.0 to 3.3 Mrad for 10⁷ spores/can and 2.9 to 3.1 Mrad for 10⁸ spores/can (Table 4). The initial spore population in each can had a much lesser effect on the MRD when estimated by extreme value techniques than by the customary methods.

Botulinal strain antagonism? The data in Table 2 disclosed an unexpected phenomenon. At 1.8 Mrad, all 100 swollen cans harbored a mixture of type A and B toxins, as anticipated. However, only 60 of these toxic cans had a
mixture of type A and B recoverable botulinum cells; 40 of the swollen toxic (type A plus B) cans contained exclusively type A viable cells and none of the cans showed solely type B cells. At 2.2 Mrad, of the 50 swollen cans only 10 possessed a mixture of type A and B toxins, whereas 33 cans contained just type A, and four cans type B, viable cells. Although 10 cans had type B toxin, only four of these cans possessed recoverable type B cells.

These data, expanded in greater detail for 1.8 and 2.2 Mrad in Table 3, did not reveal any consistent pattern to this anomaly.

**DISCUSSION**

In the past (3, 4, 6, 9, 10, 20) serious reservations have been expressed regarding the conventional practice of estimating a "12D" dose based upon the assumption that the rate of spore kill in an inoculated pack is a simple exponential (i.e., without an initial shoulder) distribution. Recently, Ross (44) analyzed the inoculated pack technique as a probabilistic model. His studies disclosed that two interrelated distribution functions, or death rates, were operating simultaneously in a radiation (or thermal) process: (i) a spore inactivation rate within a can of food and (ii) a can sterilization rate, where the replicate cans undergo sterilization as the dose increases; this rate is equal to the probability of inactivating the most resistant spore in a can of food. The traditional concept takes into account only the first distribution function and assumes it to be a simple exponential, whereas the second one has rarely, if ever, been considered. Yet the latter arises naturally as the extreme (largest) value distribution derived from the former (44) and should be reflected in the computation of an MRD process.

The rate of can sterilization with dose is obtained directly from the partial spoilage data of the inoculated pack, but the mode of spore kill in a can of food is not always obvious. To illustrate, the 2.170 to 2.228 Mrad experimental data in Table 3 were plotted as the probability of spore survival against the doses received (Fig. 2). Applying extreme value statistics, and assuming in turn the various distributions listed in Table 4, the computed results were graphed. A comparison of the derived curves (Fig. 2) indicated, at the 90% confidence level, that the conventionally used simple exponential fits the experimental data the poorest, whereas the curve obtained by assuming that spore death followed either a shifted exponential, Weibull, lognormal or normal rate fits the actual data more closely. The central limit theorem of probability suggests that the distribu-
tion is likely to be normal, not exponential, in a mixture of strains, provided that the strain resistances do not differ widely from one another. Which of the latter distribution functions actually fit best is not apparent because of an insufficient number of partial spoilage points at greater spaced increments (at least 0.1, instead of 0.01 to 0.02, Mrad units). Statistical analysis could not distinguish between these curves; hence they superimpose as practically one plot.

Interestingly, Ross also demonstrated by extreme value statistics (unpublished data) that if the rate of spore kill in an inoculated pack followed a simple exponential distribution the equation for estimating a D value should be

\[ \text{Dose} = \frac{\log M - \log (2.303n \log (n/q))}{\log (n/q)} \]

where M is the product of the spore population per can and the number of replicate cans per dose (n), and q is the number of nonspoiled cans per dose (non-swollen, nontoxic, or sterile, depending on the spoilage criterion desired). This formula is identical with that obtained by Anellis and Werkowski (9, equation 2) when they substituted the Halvorson-Ziegler (31) most-probable-number expression \( \bar{x} = 2.303a \log n/q \) for S in the Schmidt-Nank (48) relationship \( D = \text{dose} / (\log M - \log S) \), where S was defined as the number of spoiled cans per dose, which the latter authors erroneously equated with the number of surviving spores. Stumbo et al. (50) also used \( \bar{x} \) and derived a similar equation for estimating thermal D values, whereas Schmidt (46) applied \( \bar{x} \) when \( n/q = 0.5 \) so that an LD\(_{50}\) (dose at which 50% of the cans are sterile) may be utilized to compute a D value \( D = \text{LD}_{50} / \log A \cdot \log 0.69 \) where A is the spore population per can.

Ross developed formulas for estimating "12D" equivalents if the spore death rate followed a Weibull or a lognormal (44), as well as for a normal or a shifted exponential (unpublished data), distribution. Table 4 shows that all four of these distribution functions gave MRD values of 3.0 to 3.2 Mrad if the spore density level is assumed to be 10\(^7\) of a single most resistant strain per can, and 2.9 to 3.0 Mrad on the basis of a mixture of 10 strains containing 10\(^7\) 

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**FIG. 2.** Comparison of several common probability distribution models for radiation survival of C. botulinum spores in beef. Symbols: ○, experimental data; dotted line, simple exponential; solid line, shifted exponential, Weibull, lognormal, normal. All distributions were computed by extreme value statistics. F is the probability of spore inactivation at dose 4.
speres/can. These close results are related to the fact that Fig. 2 has only a single curve for the latter four rates of death. If the computation is made by the Anellis-Werkowski (10) normal distribution, the MRD becomes 3.3 and 3.1 Mrad, respectively (Table 4). Thus, on the average, the radappertization dose for beef is 3.0 Mrad. However, if the sterilizing dose is estimated by the prescribed simple exponential equations (9, equations 1 and 3), the "12D" is 4.3 \((10^6\) spores/can) and 3.7 \((10^5\) spores/can) Mrad, respectively, noticeably higher than by the other calculations; the same values were obtained by using the extreme value method of estimating the simple exponential MRD (9, equation 2). Furthermore, the number of spores per can had a much greater influence on the MRD (Table 4), although this should not occur if the spore death rate is truly exponential. It is not surprising that the same "12D" results were secured by the three simple exponential estimates because (i) the inoculated pack had only one significant partial spoilage point (2.2 Mrad), and (ii) when the number of sterile cans are near the LD\(_{50}\) point then the number of surviving organisms per can is close to one (0.69) and hence becomes equal with log \(S\) (the number of spoiled cans as defined by Schmidt and Nank [48]).

The data in Tables 2 and 3 indicate that some form of bacterial antagonism occurred in the mixed \(C.\ botulinum\) culture in the cans of beef, the type B strains being apparently more susceptible to the competitive microenvironment than the type A strains. Other investigators (15, 16, 19, 29, 33, 42, 48) have used a pooled spore suspension of type A and B botulinal strains in their inoculated pack studies, but, with the exception of Schmidt and Nank (48), they assayed the foods for toxin with a mixture of type A and B antiserum; hence they did not observe the apparent antibacterial type of phenomenon found by us. Schmidt and Nank (48), however, tested their products with type-specific antitoxins A and B; they reported that spoilage was due exclusively to type A organisms and assumed that the type B portion of the irradiated pooled inoculum had not produced any irradiation survivors.

Schmidt and Nank (48) may have been correct in assuming greater irradiation sensitivity for their two type B botulinal strains than for one (or more) of their type A strains in their mixed inoculum. Our data did not support that sort of simple relationship, because we found that meat samples irradiated to 2.2 Mrad (the highest sublethal dose used) contained only type B toxin and/or type A viable cells, or solely type A toxin and/or type A viable cells, or a mixture of type A and B toxins and recoverable cells (Table 2).

Our results (Tables 2 and 3) reflect a complex phenomenon which may be due to one or more series of events occurring either simultaneously or sequentially: (i) the secretion into the food substrate by one or more strains of \(C.\ botulinum\) of a botulinin-like substance, a bacteriocin, which is usually antagonistic against a limited number of strains of the same or closely related species of organism. Botulin production by type A, B, C, and E strains has been reported previously (1, 12, 22, 37, 40). A preliminary investigation in our laboratory seemed to indicate such a possibility with our strains. (ii) The survivors of irradiation, which had undergone various degrees of injury, had different rates of spore repair, germination, and outgrowth, and consequently the more rapidly proliferating cells suppressed cells with a slower growth rate and a greater amount of irradiation damage. And (iii) proteolytic enzymes, secreted by the growth of some of the surviving strains, destroyed relatively low levels of preformed toxins elaborated by slower metabolizing cells.

In practice, if food prepared for irradiation processing were contaminated by \(C.\ botulinum\) spores, one could expect the contaminants to include strains with differing germination and outgrowth cycles as well as to possess various biochemical capabilities and a variety of radiation sensitivities. Hence our use of an inoculum with a mixture of strains in our inoculated pack study probably typified real situations more closely than the use of pure culture inocula.

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