

# Radiation Sterilization of Prototype Military Foods

## III. Pork Loin

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Ten lots of pork loin, packed in cans, were inoculated with approximately  $10^6$  *Clostridium botulinum* spores per can. Each lot was seeded with a different strain; five type A and five type B strains were used. The pack comprised 5,690 cans, including controls, and contained about  $10^9$  spores per dose. The cans were irradiated with  $Co^{60}$  in the range of 0 to 5.0 Mrad (0.5 Mrad increments) at 5 to 25 C, incubated for 6 months at 30 C, and examined for swelling, toxicity, and recoverable *C. botulinum*. The minimal experimental sterilizing dose (ESD) based on nonswollen, nontoxic, but nonsterile end points was  $2.5 < ESD \leq 3.0$  Mrad, and based on nonspoilable sterile cans was  $3.5 < ESD \leq 4.0$  Mrad. The theoretical minimal radiation dose (MRD), the 12D equivalent, varied with the method of computation: 4.74,  $4.33 \pm 0.17$ , and 4.19 to 4.99 Mrad were obtained by the Weibull, Spearman-Kärber, and Schmidt-Nank techniques, respectively. Calculation of D and MRD values by the conventional Schmidt-Nank method produced increasing values with rising dosage; this finding was compared with the data derived by the other two methods of calculation. Suggestions for estimating the MRD of a prototype radiation process are offered.

Development of a prototype radiation sterilization process, adaptable for commercial production, has been reported by the U. S. Army Natick Laboratories for bacon (2) and ham (1). Irradiated bacon has already been produced on a commercial trial basis. Research has progressed to a point where fresh pork could be scheduled as the next food for radioprocess development.

Several inoculated pack studies involving irradiation of noncured pork have been reported in the scientific literature. Pratt et al. (10) irradiated, at low temperature (-18 to -28 C), ground parboiled pork containing a *Clostridium botulinum* mixture of five type A and five type B strains, totaling about  $10^6$  spores per can, or  $10^8$  spores per dose. They observed that 3.72 Mrad prevented visible spoilage up to 12 months of incubation at 30 C, but they did not provide complete data on the presence or absence of *C. botulinum* toxin or recoverable *C. botulinum* in the flat cans; however, some spores (0.04 per g) were found in nonincubated cans subjected to 3.72 Mrad, but none were recovered at 2.98, 3.26, 3.44, and 4.19 Mrad, the highest dose used. Schmidt and Nank (13), using a mixture of three type A and two type B strains totaling about  $10^8$  spores per dose, obtained nonswollen, nontoxic, sterile cans when irradiated to 2.70 Mrad at 26.7 C and incubated for 12 to 15 months at 29.4 C. The minimal radia-

tion dose (MRD), or the equivalent theoretical 12D dose, was 3.30 to 3.60 Mrad. Wheaton et al. (15) found that  $2 \times 10^8$ - $9 \times 10^8$  spores per can of ground pork, or  $6 \times 10^8$ - $27 \times 10^9$  spores per dose, of their most radioresistant *C. botulinum* strain was reduced to 0.4% of the original inoculum when irradiated at -29 C to 1.7 Mrad, the highest dose tested. Since product incubation of the inoculated cans was not carried out, partial spoilage or end point data were not available, hence, computation of a 12D dose seems unjustified. Ingram and Thornley (7) irradiated minced pork, infected with about  $10^6$  *C. botulinum* spores per can ( $3 \times 10^6$  spores per dose), up to 2.0 Mrad at both 0 C and -75 C. Survival count data indicated that 0.004% of the initial inoculum withstood 2.0 Mrad at 0 C, and that 0.02% of the spores survived this dose at -75 C irradiation. The MRD at the higher temperature was estimated to be 4.69 to 4.71 Mrad and at the lower temperature was 5.37 to 6.27 Mrad. The authors cautioned, however, that their calculations were based upon assumptions that were not completely sound. All of the above studies were conducted with spent fuel rods.

A noninoculated raw pork pack was irradiated with electrons to 2.0 Mrad at 18 C and to 5.0 Mrad at -75 C by Coleby et al. (4). Neither dose produced a sterile product; 5 of 65 cans exposed to

2.0 Mrad spoiled, whereas 1 of 140 cans receiving 5.0 Mrad apparently harbored streptococci. The investigators pointed out that the doses delivered were not uniformly absorbed by the meat, the dose deficiency being as high as 25% in some instances; hence, the results "should not occasion alarm."

A single case of botulism, implicating commercially prepared pork and beans, occurred in 1906 in the U.S.A.; since that incident, no additional outbreaks associated with commercial pork or pork products were reported through 1963 (9). The Morbidity and Mortality Weekly Reports for 1964, 1966, 1967, and 1968 did not incriminate pork in any botulism outbreaks in the U.S.A. The 1965 reports listed two outbreaks: one allegedly involving canned pork and beans, and the second, pork luncheon meat which had been prepared from canned pork (sliced, vacuum-sealed in plastic pouches and held under refrigeration in a supermarket). Prompt laboratory investigations by the National Communicable Disease Center (NCDC) of the U. S. Public Health Service failed to implicate these pork products as a cause of botulism (NCDC, *personal communication*). Thus, commercial pork products have enjoyed a botulism-free safety record for at least 60 years.

Over a period of 12 years (1957 through 1968), a total of 1,472 noninoculated samples of fresh pork were preserved in our laboratory by ionizing radiation. The product, packaged either in metal cans or in flexible pouches and exposed to doses ranging from 0.6 to 6.0 Mrad, was examined for *C. botulinum* toxin before evaluation by consumer taste panels. None of these samples was toxic to mice.

In spite of this excellent public health record, it must be assumed that fresh pork can become contaminated with *C. botulinum* spores. Therefore, a commercial radiation sterilization process, to be microbiologically safe, must be capable of destroying this organism in considerably higher numbers than would normally be found in the product.

This communication presents results applicable to the establishment of a commercial radiation sterilization process for fresh pork.

#### MATERIALS AND METHODS

**Food preparation.** The pork used complied with Federal Specification PP-P-0057b (10 October 1963) under the following description: loin, fresh chill, U. S. no. 1, 6 1/2 to 8 1/2 lb boneless weight. The loins were trimmed, packed tightly into 7.6-cm diameter casing (no. 3 1/2, regenerated cellulose; Visking Co., Div. of Union Carbide Corp., Chicago, Ill.), and the ends clipped together. The encased loins were then enzyme-inactivated in a steam retort to an in-

ternal temperature of 71.1 to 73.9 C (5 psi for ca. 45 min), wrapped in clean paper, and refrigerated overnight at 0 to 3.3 C. The loins were removed from the casings and the meat was cut into approximately 4.5 cm, 100  $\pm$  10 g slices. The slices were packed snugly into enamel metal cans (300 by 200 cm), and covered with lids, leaving a 0.5 cm headspace per can. Sanitary precautions were followed throughout the handling procedure, including prior autoclaving of the cans and lids for 10 min at 5 psi.

**Test organisms, inoculation, irradiation, assays.** Five type A and five type B strains of *C. botulinum* spores were used. The procedures for inoculation of the meat, irradiations, and assays for spoilage were previously described (1).

This pack contained 545 cans per lot and a total of 5,690 cans with controls; 20 replicate cans were irradiated at each dose level in the range 0 to 3.0 Mrad in 0.5-Mrad increments, and 100 replicate cans were similarly irradiated in the range 3.5 to 5.0 Mrad.

**Calculation of radiation resistance of *C. botulinum* spores.** Radiation *D* values, the accepted index of microbial resistance, which assumes exponential death of the test organism in an inoculated pack, were computed by the conventional Schmidt-Nank method (13). In addition, the Spearman-Kärber technique (3) was used to estimate LD<sub>50</sub> values which were converted to *D* values (11). The MRD was calculated as  $D \times 12$ . The Weibull analysis (3) for determining the 12*D* equivalent was used where applicable. The advantages of the two latter methods were fully discussed elsewhere (3).

#### RESULTS

**Initial spore population.** The number of *C. botulinum* spores inoculated into each can and the total spore population per strain are presented in Table 1. Each radiation dose in the range 0 to 3.0 Mrad was challenged by a total load of  $1.3 \times 10^9$  spores, and in the range 3.5 to 5.0 Mrad, by  $6.3 \times 10^9$  spores—massive *C. botulinum* densities not found in raw meats (1).

**Experimental sterilizing dose (ESD).** Table 2 details the spoilage data for each of the 10 strains at all radiation levels under the three test criteria: swelling, toxicity, and viable *C. botulinum*. Table 3 combines the data and accumulates the spoilage levels among the 10 strains.

Lots of 1,000 cans per dose, containing a total of  $6.3 \times 10^9$  spores and irradiated to 4.0 Mrad or higher, were nonswollen, nontoxic, and sterile (Table 3). A similar lot of 1,000 cans exposed to 3.5 Mrad was entirely free from swelling and toxic spoilage; only 4 of the 1,000 cans contained viable *C. botulinum* (strains 33A, 77A, and 40B). A 200-can lot, containing a total *C. botulinum* spore population of  $1.3 \times 10^9$ , and subjected to 3.0 Mrad, was also free from swelling and toxic spoilage; only 2 of the 200 cans harbored dormant *C. botulinum* (strains 33A and 62A). A similar 200-can lot irradiated to 2.5 Mrad showed just

TABLE 1. Inoculum levels of *Clostridium botulinum* spores in pork loin

| Strain no. | Radiation dose (Mrad) | Spores per can         | No. of cans per dose | Total spore inoculum per strain <sup>a</sup> |
|------------|-----------------------|------------------------|----------------------|--|
| 33A        | 0-3.0 <sup>b</sup>    | 5.22 × 10 <sup>6</sup> | 20                   | 1.04 × 10 <sup>8</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 5.22 × 10 <sup>8</sup>                       |
| 36A        | 0-3.0                 | 0.99 × 10 <sup>6</sup> | 20                   | 1.98 × 10 <sup>7</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 0.99 × 10 <sup>8</sup>                       |
| 62A        | 0-3.0                 | 1.37 × 10 <sup>6</sup> | 20                   | 2.74 × 10 <sup>7</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 1.37 × 10 <sup>8</sup>                       |
| 77A        | 0-3.0                 | 6.10 × 10 <sup>6</sup> | 20                   | 1.22 × 10 <sup>8</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 6.10 × 10 <sup>8</sup>                       |
| 12885A     | 0-3.0                 | 4.94 × 10 <sup>6</sup> | 20                   | 9.88 × 10 <sup>7</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 4.94 × 10 <sup>8</sup>                       |
| 9B         | 0-3.0                 | 8.73 × 10 <sup>6</sup> | 20                   | 1.75 × 10 <sup>8</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 8.73 × 10 <sup>8</sup>                       |
| 40B        | 0-3.0                 | 2.36 × 10 <sup>6</sup> | 20                   | 4.72 × 10 <sup>7</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 2.36 × 10 <sup>8</sup>                       |
| 41B        | 0-3.0                 | 9.10 × 10 <sup>6</sup> | 20                   | 1.82 × 10 <sup>8</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 9.10 × 10 <sup>8</sup>                       |
| 51B        | 0-3.0                 | 1.57 × 10 <sup>7</sup> | 20                   | 3.14 × 10 <sup>8</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 1.57 × 10 <sup>9</sup>                       |
| 53B        | 0-3.0                 | 8.36 × 10 <sup>6</sup> | 20                   | 1.67 × 10 <sup>8</sup>                       |
|            | 3.5-5.0               |                        | 100                  | 8.36 × 10 <sup>8</sup>                       |

<sup>a</sup> Accumulated spore inoculum for 10 strains per dose: 20 can lots, 1.26 × 10<sup>9</sup>; 100 can lots, 6.29 × 10<sup>9</sup>.

<sup>b</sup> Doses increase in 0.5 Mrad increments.

six swollen toxic cans and contained viable *C. botulinum* organisms (strains 33A and 53B). An additional 23 cans had dormant *C. botulinum* (strains 33A, 62A, 77A, 12885A, 9B, 40B, 41B, and 53B), but were unspoiled. The ratio of spoilage at 2.5 Mrad is notably low, considering the extremely high spore inoculum. Radiation doses below 2.5 Mrad yielded substantial amounts of spoilage.

The ESD, based on the three spoilage criteria, are indicated in Table 4. Nonswollen, nontoxic, but nonsterile pork loin was obtained with 2.5 < ESD ≤ 3.0 Mrad with the inactivation of strains 33A and 53B; but, to attain nonspoiled pork free from dormant *C. botulinum* required the inactivation of strains 33A, 77A, and 40B with 3.5 < ESD ≤ 4.0 Mrad.

MRD. Partial spoilage data (Table 2) were used to calculate Schmidt-Nank and Spearman-Kärber radiation *D* values for the three spoilage

TABLE 2. Effect of Co<sup>60</sup> irradiation on spoilage of pork loin inoculated with *Clostridium botulinum* spores

| Strain no. | Radiation dose (Mrad) | No. of cans of pork loin |         |            |                                 | <i>D</i> values <sup>a</sup> based on viable <i>C. botulinum</i> (Mrad) |
|------------|-----------------------|--------------------------|---------|------------|---------------------------------|---|
|            |                       | Tested                   | Swollen | With toxin | With viable <i>C. botulinum</i> |   |
| 33A        | 0-2.0 <sup>b</sup>    | 20 <sup>c</sup>          | 20      | 20         | 20                              | 0.349<br>0.374<br>0.416   |
|            | 2.5                   | 20                       | 2       | 2          | 7                               |   |
|            | 3.0                   | 20                       | 0       | 0          | 1                               |   |
|            | 3.5                   | 100                      | 0       | 0          | 2                               |   |
|            | 4.0-5.0               | 100 <sup>d</sup>         | 0       | 0          | 0                               |   |
| 36A        | 0-1.5                 | 20                       | 20      | 20         | 20                              | 0.310   |
|            | 2.0                   | 20                       | 4       | 4          | 7                               |   |
|            | 2.5, 3.0              | 20                       | 0       | 0          | 0                               |   |
|            | 3.5-5.0               | 100                      | 0       | 0          | 0                               |   |
| 62A        | 0-1.5                 | 20                       | 20      | 20         | 20                              | 0.325<br>0.336<br>0.403   |
|            | 2.0                   | 20                       | 16      | 16         | 19                              |   |
|            | 2.5                   | 20                       | 0       | 0          | 1                               |   |
|            | 3.0                   | 20                       | 0       | 0          | 1                               |   |
|            | 3.5-5.0               | 100                      | 0       | 0          | 0                               |   |
| 77A        | 0-2.0                 | 20                       | 20      | 20         | 20                              | 0.321<br>0.398  |
|            | 2.5                   | 20                       | 0       | 0          | 2                               |   |
|            | 3.0                   | 20                       | 0       | 0          | 0                               |   |
|            | 3.5                   | 100                      | 0       | 0          | 1                               |   |
|            | 4.0-5.0               | 100                      | 0       | 0          | 0                               |   |
| 12885A     | 0-1.5                 | 20                       | 20      | 20         | 20                              | 0.282<br>0.338  |
|            | 2.0                   | 20                       | 6       | 6          | 8                               |   |
|            | 2.5                   | 20                       | 0       | 0          | 4                               |   |
|            | 3.0                   | 20                       | 0       | 0          | 0                               |   |
|            | 3.5-5.0               | 100                      | 0       | 0          | 0                               |   |
| 9B         | 0-1.5                 | 20                       | 20      | 20         | 20                              | 0.287<br>0.335  |
|            | 2.0                   | 20                       | 17      | 17         | 19                              |   |
|            | 2.5                   | 20                       | 0       | 0          | 6                               |   |
|            | 3.0                   | 20                       | 0       | 0          | 0                               |   |
|            | 3.5-5.0               | 100                      | 0       | 0          | 0                               |   |
| 40B        | 0-1.5                 | 20                       | 20      | 20         | 20                              | 0.290<br>0.339<br>0.418   |
|            | 2.0                   | 20                       | 2       | 2          | 6                               |   |
|            | 2.5                   | 20                       | 0       | 0          | 2                               |   |
|            | 3.0                   | 20                       | 0       | 0          | 0                               |   |
|            | 3.5                   | 100                      | 0       | 0          | 1                               |   |
|            | 4.0-5.0               | 100                      | 0       | 0          | 0                               |   |

<sup>a</sup> Computed by the Schmidt-Nank equation (12).

<sup>b</sup> Doses increase in 0.5 Mrad increments.

<sup>c</sup> Twenty replicate cans per dose.

<sup>d</sup> One hundred replicate cans per dose.

TABLE 2—Continued

| Strain no. | Radiation dose (Mrad) | No. of cans of pork loin |         |            |                                 | <i>D</i> values <sup>a</sup> based on viable <i>C. botulinum</i> (Mrad) |
|------------|-----------------------|--------------------------|---------|------------|---------------------------------|---|
|            |                       | Tested                   | Swollen | With toxin | With viable <i>C. botulinum</i> |   |
| 41B        | 0-1.5                 | 20                       | 20      | 20         | 20                              | 0.286<br>0.303  |
|            | 2.0                   | 20                       | 20      | 20         | 19                              |   |
|            | 2.5                   | 20                       | 0       | 0          | 1                               |   |
|            | 3.0                   | 20                       | 0       | 0          | 0                               |   |
|            | 3.5-5.0               | 100                      | 0       | 0          | 0                               |   |
| 51B        | 0-0.5                 | 20                       | 20      | 20         | 20                              | 0.132<br>0.192<br>0.253   |
|            | 1.0                   | 20                       | 2       | 0          | 8                               |   |
|            | 1.5                   | 20                       | 0       | 0          | 5                               |   |
|            | 2.0                   | 20                       | 0       | 0          | 4                               |   |
|            | 2.5, 3.0              | 20                       | 0       | 0          | 0                               |   |
|            | 3.5-5.0               | 100                      | 0       | 0          | 0                               |   |
| 53B        | 0-2.0                 | 20                       | 20      | 20         | 20                              | 0.336   |
|            | 2.5                   | 20                       | 4       | 4          | 6                               |   |
|            | 3.0                   | 20                       | 0       | 0          | 0                               |   |
|            | 3.5-5.0               | 100                      | 0       | 0          | 0                               |   |

TABLE 3. Cumulative spoilage data of irradiated pork loin inoculated with *Clostridium botulinum* spores

| Radiation dose (Mrad) | Total spore population <sup>a</sup> | No. of cans of pork loin |         |            |                                 |
|-----------------------|-------------------------------------|--------------------------|---------|------------|---------------------------------|
|                       |                                     | Tested                   | Swollen | With toxin | With viable <i>C. botulinum</i> |
| 0                     | 0                                   | 100                      | 100     | 0          | 0                               |
| 0                     | $1.26 \times 10^9$                  | 200                      | 200     | 200        | 200                             |
| 0.5                   | $1.26 \times 10^9$                  | 200                      | 200     | 200        | 200                             |
| 1.0                   | $1.26 \times 10^9$                  | 200                      | 182     | 180        | 188                             |
| 1.5                   | $1.26 \times 10^9$                  | 200                      | 180     | 180        | 185                             |
| 2.0                   | $1.26 \times 10^9$                  | 200                      | 125     | 125        | 142                             |
| 2.5                   | $1.26 \times 10^9$                  | 200                      | 6       | 6          | 29                              |
| 3.0                   | $1.26 \times 10^9$                  | 200                      | 0       | 0          | 2                               |
| 3.5                   | $6.29 \times 10^9$                  | 1000                     | 0       | 0          | 4                               |
| 4.0                   | $6.29 \times 10^9$                  | 1000                     | 0       | 0          | 0                               |
| 4.5                   | $6.29 \times 10^9$                  | 1000                     | 0       | 0          | 0                               |
| 5.0                   | $6.29 \times 10^9$                  | 1000                     | 0       | 0          | 0                               |

<sup>a</sup> Information pooled from 10 strains.

criteria for each *C. botulinum* strain. The results are indicated in Table 5 as comparative radioresistances of the ten strains.

On the basis of viable *C. botulinum* *D* values, the type A spore strains appeared to be of somewhat higher radioresistance as a group than the type B strains, although there was overlapping. The Spearman-Kärber order of resistance, using the upper confidence intervals, was 62A = 33A >

53B > 9B > 77A > 36A > 41B > 40B = 12885A > 51B (Table 5). The comparative order of strain resistances could not be determined, however, by the Schmidt-Nank assay due to a nonuniform minimum-maximum spread of the *D* values within the strains.

With one exception (51B), identical *D* values were obtained for swelling and toxic spoilage (Table 5); strain 51B had a somewhat higher *D* value for swelling than for toxin production. Viable *C. botulinum* data yielded the highest *D* values, as expected.

The data in Table 2 reveal an interesting phenomenon; the *D* values computed by the Schmidt-Nank technique increased with increasing dosage for every *C. botulinum* strain which gave more than one partial spoilage end point. Hence, the *D* values in Table 5 indicate the minimum-maximum range for the individual strains involved.

Theoretical 12*D* values for the three types of spoilage are recorded in Table 6. The Schmidt-Nank calculations indicate that visible and toxic spoilage of the pork loin, based on single partial spoilage end points, may be prevented by an MRD of about 3.94 Mrad. The MRD which will render this product sterile is not clear, due to the variable spreads of the computed values; the minimum-maximum range differed from a low of 0.2 Mrad (strain 41B) to as high as 1.5 Mrad (strain 40B), depending, at least in part, on the number of partial spoilage end points involved in the calculations.

The Spearman-Kärber computations indicate that visible and toxic spoilage may be eliminated by an MRD of approximately  $4.09 \pm 0.17$  Mrad (strain 62A), in good agreement with the Schmidt-Nank data above; to obtain sterility, an MRD of about  $4.33 \pm 0.17$  Mrad (strain 62A or 33A) would be needed.

The partial spoilage data in Table 2 were inadequate for a Weibull assessment of an MRD.

The pooled information in Table 3 was also employed to compute MRD values for the three spoilage criteria, but the results provided by strain 51B were deleted from the totals, since 51B was atypically sensitive among the 10 strains (Table 2). Its observed dose response began at least 1.0 Mrad lower than the remainder of the strains. Moreover, its inclusion in a Weibull assay created an irrational plot ( $\alpha$   $\beta$ -value > 5), possibly resulting from experimental error or an inherent distinctive variability in the organism, or both. Thus, its use in the pooled data had undue influence on the remainder of the results. The omission of strain 51B reduced the six spoilage end points to those of the four highest doses, thus producing increased computed MRD values.

Table 7 compares the MRD values derived

TABLE 4. Experimental sterilizing dose<sup>a</sup> based on different criteria of spoilage by *Clostridium botulinum* spores in irradiated pork loin

| Strain no. | Minimal radiation dose to eliminate <sup>b</sup> |                 |                            |
|------------|--|-----------------|----------------------------|
|            | Swelling   | Toxin           | Viable <i>C. botulinum</i> |
| 33A        | 2.5 < ESD ≤ 3.0                                  | 2.5 < ESD ≤ 3.0 | 3.5 < ESD ≤ 4.0            |
| 36A        | 2.0 < ESD ≤ 2.5                                  | 2.0 < ESD ≤ 2.5 | 2.0 < ESD ≤ 2.5            |
| 62A        | 2.0 < ESD ≤ 2.5                                  | 2.0 < ESD ≤ 2.5 | 3.0 < ESD ≤ 3.5            |
| 77A        | 2.0 < ESD ≤ 2.5                                  | 2.0 < ESD ≤ 2.5 | 3.5 < ESD ≤ 4.0            |
| 12885A     | 2.0 < ESD ≤ 2.5                                  | 2.0 < ESD ≤ 2.5 | 2.5 < ESD ≤ 3.0            |
| 9B         | 2.0 < ESD ≤ 2.5                                  | 2.0 < ESD ≤ 2.5 | 2.5 < ESD ≤ 3.0            |
| 40B        | 2.0 < ESD ≤ 2.5                                  | 2.0 < ESD ≤ 2.5 | 3.5 < ESD ≤ 4.0            |
| 41B        | 2.0 < ESD ≤ 2.5                                  | 2.0 < ESD ≤ 2.5 | 2.5 < ESD ≤ 3.0            |
| 51B        | 1.0 < ESD ≤ 1.5                                  | 0.5 < ESD ≤ 1.0 | 2.0 < ESD ≤ 2.5            |
| 53B        | 2.5 < ESD ≤ 3.0                                  | 2.5 < ESD ≤ 3.0 | 2.5 < ESD ≤ 3.0            |

<sup>a</sup> Experimental sterilizing dose, ESD.

<sup>b</sup> Expressed in Mrad values.

TABLE 5. Comparative radioresistance of strains of *Clostridium botulinum* spores in pork loin

| Strain no. | Radiation <i>D</i> values based on |                              |                            |                 |
|------------|------------------------------------|------------------------------|----------------------------|-----------------|
|            | Spoilage <sup>a</sup>              |                              | Viable <i>C. botulinum</i> |                 |
|            | Schmidt-Nank <sup>b</sup>          | Spearman-Kärber <sup>c</sup> | Schmidt-Nank               | Spearman-Kärber |
| 33A        | 0.324                              | 0.334 ± 0.011 <sup>d</sup>   | 0.349-0.416 <sup>e</sup>   | 0.358 ± 0.017   |
| 36A        | 0.299                              | 0.301 ± 0.014                | 0.310                      | 0.313 ± 0.017   |
| 62A        | 0.321                              | 0.341 ± 0.014                | 0.325-0.403                | 0.361 ± 0.014   |
| 77A        | < 0.309 <sup>f</sup>               | < 0.328 <sup>f</sup>         | 0.321-0.398                | 0.331 ± 0.010   |
| 12885A     | 0.277                              | 0.277 ± 0.046                | 0.282-0.338                | 0.299 ± 0.020   |
| 9B         | 0.285                              | 0.306 ± 0.011                | 0.287-0.335                | 0.334 ± 0.016   |
| 40B        | 0.271                              | 0.276 ± 0.010                | 0.290-0.418                | 0.299 ± 0.019   |
| 41B        | < 0.295 <sup>f</sup>               | < 0.320 <sup>f</sup>         | 0.286-0.303                | 0.316 ± 0.010   |
| 51B        | 0.122 <sup>g</sup>                 | 0.109 ± 0.009 <sup>h</sup>   | 0.132-0.253                | 0.159 ± 0.023   |
| 53B        | 0.328                              | 0.332 ± 0.013                | 0.336                      | 0.339 ± 0.014   |

<sup>a</sup> Swelling and toxicity.

<sup>b</sup> Computed by the Schmidt-Nank equation (12):  $D = \text{Mrad}/(\log M - \log S)$ .

<sup>c</sup> Computed by the Spearman-Kärber equation:

$$LD_{50} = t_w + \frac{d}{2} - d \sum_{i=1}^u P_i$$

followed by  $D = LD_{50}/(\log A - \log 0.69)$  by Schmidt (3).

<sup>d</sup> Confidence intervals computed at the 95% level of probability.

<sup>e</sup> Minimum-maximum range of *D* values.

<sup>f</sup> Insufficient data; assumed 1/20 spoiled can at 2.5 Mrad for computational purposes only.

<sup>g</sup> *D* value for swelling only; the *D* value for toxic spoilage was < 0.118.

<sup>h</sup> *D* value for swelling only; the *D* value for toxic spoilage was < 0.099.

from the pooled results (minus strain 51B) by the three statistical procedures. On the basis of two partial spoilage end points, the Schmidt-Nank and Spearman-Kärber estimates for obtaining nonspoilable nonsterile pork were in good agreement with MRD values of 3.49 to 3.66 Mrad and  $3.69 \pm 0.06$  Mrad, respectively. To achieve sterility of the nonspoilable product, an MRD of about  $3.88 \pm 0.22$  Mrad was required, according

to the Spearman-Kärber calculation; the Schmidt-Nank technique, however, based on four partial spoilage end points, yielded too wide a minimum-maximum MRD range (3.51 to 4.63 Mrad) to be useful.

The Weibull analysis of the cumulative viable *C. botulinum* data suggested an MRD of 4.74 Mrad, which agreed with the Spearman-Kärber maximum 12*D* estimate (4.50 Mrad) in Table 6.

TABLE 6. Minimal radiation dose for pork loin inoculated with *Clostridium botulinum* spores

| Strain no. | Total spores per dose | ESD <sup>a</sup>            | Computed 12 D dose <sup>b</sup> based on |                 |                            |                 |
|------------|-----------------------|-----------------------------|--|-----------------|----------------------------|-----------------|
|            |                       |                             | Swelling and toxin                       |                 | Viable <i>C. botulinum</i> |                 |
|            |                       |                             | Schmidt-Nank                             | Spearman-Kärber | Schmidt-Nank               | Spearman-Kärber |
| 33A        | $5.22 \times 10^8$    | $3.5 < \text{ESD} \leq 4.0$ | 3.89                                     | $4.01 \pm 0.13$ | $4.19-4.99^d$              | $4.30 \pm 0.20$ |
| 36A        | $1.98 \times 10^7$    | $2.0 < \text{ESD} \leq 2.5$ | 3.59                                     | $3.61 \pm 0.17$ | 3.72                       | $3.76 \pm 0.20$ |
| 62A        | $1.37 \times 10^8$    | $3.0 < \text{ESD} \leq 3.5$ | 3.85                                     | $4.09 \pm 0.17$ | $3.90-4.84$                | $4.33 \pm 0.17$ |
| 77A        | $6.10 \times 10^8$    | $3.5 < \text{ESD} \leq 4.0$ | $< 3.71^c$                               | $< 3.94^c$      | $3.85-4.78$                | $3.97 \pm 0.12$ |
| 12885A     | $9.88 \times 10^7$    | $2.5 < \text{ESD} \leq 3.0$ | 3.32                                     | $3.32 \pm 0.55$ | $3.38-4.06$                | $3.59 \pm 0.24$ |
| 9B         | $1.75 \times 10^8$    | $2.5 < \text{ESD} \leq 3.0$ | 3.42                                     | $3.67 \pm 0.13$ | $3.44-4.02$                | $4.01 \pm 0.19$ |
| 40B        | $2.36 \times 10^8$    | $3.5 < \text{ESD} \leq 4.0$ | 3.25                                     | $3.31 \pm 0.12$ | $3.48-5.02$                | $3.59 \pm 0.23$ |
| 41B        | $1.82 \times 10^8$    | $2.5 < \text{ESD} \leq 3.0$ | $< 3.54^c$                               | $< 3.84^c$      | $3.43-3.64$                | $3.79 \pm 0.12$ |
| 51B        | $3.14 \times 10^8$    | $2.0 < \text{ESD} \leq 2.5$ | 1.46                                     | $1.31 \pm 0.11$ | $1.58-3.04$                | $1.91 \pm 0.28$ |
| 53B        | $1.67 \times 10^8$    | $2.5 < \text{ESD} \leq 3.0$ | 3.94                                     | $3.98 \pm 0.16$ | 4.03                       | $4.07 \pm 0.17$ |

<sup>a</sup> ESD, experimental sterilizing dose, expressed in Mrad values; flat, nontoxic, sterile cans.

<sup>b</sup> Decimation through 12-log cycles of initial spore population by the equation  $D \times 12$ ; expressed in Mrad values.

<sup>c</sup> Insufficient data; assumed 1/20 spoiled can at 2.5 Mrad for computational purposes only.

<sup>d</sup> Minimum-maximum range of minimal radiation dose values.

TABLE 7. Minimal radiation dose for pork loin based on cumulative spoilage data<sup>a</sup> by different methods of computation

| Method of computation | Criterion for <i>Clostridium botulinum</i> spoilage |                 |                                 |
|-----------------------|---|-----------------|---------------------------------|
|                       | Swelling  | Toxicity        | Recoverable <i>C. botulinum</i> |
| Schmidt-Nank          | $3.49-3.66^b$                                       | $3.49-3.66^b$   | $3.51-4.63^b$                   |
| Spearman-Kärber       | $3.69 \pm 0.06$                                     | $3.69 \pm 0.06$ | $3.88 \pm 0.22$                 |
| Weibull               | $-^c$   | $-^c$           | 4.74                            |

<sup>a</sup> Data in Table 4 were used for calculations, but with strain 51B deleted from the totals for having atypical resistance among the 10 test strains.

<sup>b</sup> Minimum-maximum range of minimal radiation dose values.

<sup>c</sup> Insufficient data for computation.

Fig. 1B appears to approximate an exponential plot, although we are uncertain of the death kinetics of the individual nine strains.

### DISCUSSION

An inoculated cured ham pack (1), handled and irradiated in a manner identical to the present pack, was found to have  $D$  values which varied directly with radiation dose when computed by the Schmidt-Nank equation (3). By definition,  $D$  values should remain approximately constant regardless of dose. A Weibull analysis of the partial spoilage data of individual and pooled strains disclosed that the *C. botulinum* mode of death in the ham followed a normal distribution. Since the Schmidt-Nank formula is based on an assumed exponential death, it was thought that the explanation for the changing  $D$  values lay with the misapplication of the Schmidt-Nank tech-

nique. It was also believed that the curing salt inhibitors in the ham could have masked or confused the bacterial radiation response.

A Weibull treatment of the pooled partial spoilage data of the pork loin pack (Table 3), but in this instance omitting the results of strain 51B from the pack, seemed to suggest that the death kinetics of the *C. botulinum* spores was approaching an exponential form (Fig. 1B). Unfortunately, this intimation could not be verified by a Weibull analysis of individual strain data. However, the pork used was a fresh, uncured product, yet the Schmidt-Nank  $D$  values rose with increasing dosage both with the cumulative and the individual strain data. Table 2 illustrates this phenomenon with the viable *C. botulinum* spoilage data. Examination by the Schmidt-Nank procedure of other inoculated pack irradiation studies reported in the literature revealed similar  $D$  value

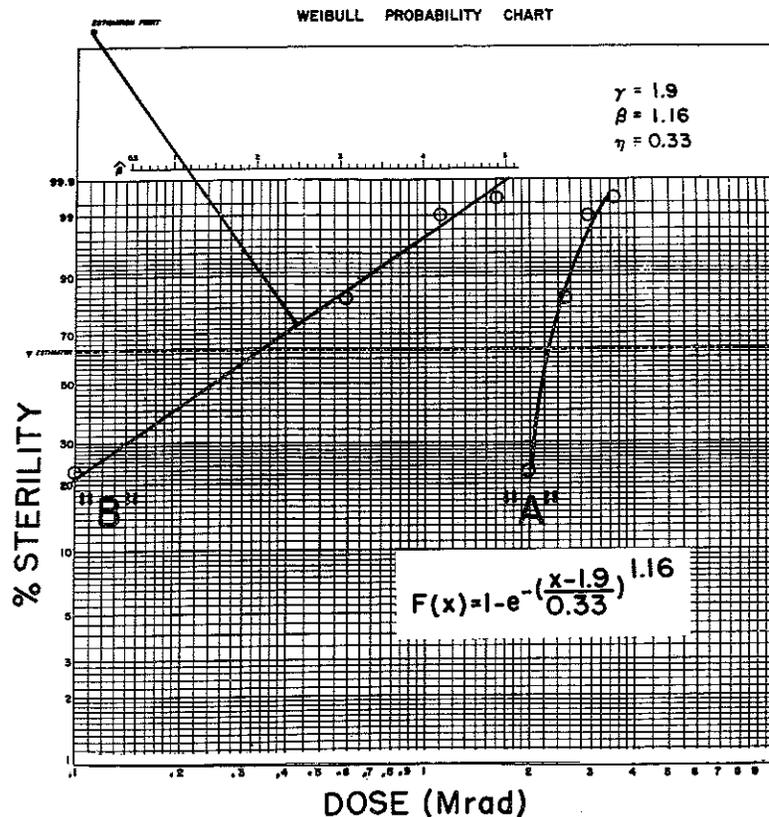


FIG. 1. Radiation survival of *C. botulinum* spores in pork loin. Cumulative data for nine strains. Strain 51B was deleted from the totals for being atypical in resistance among the 10 strains. Curve "A" is the initial plot; curve "B" is the adjusted plot from which the above equation is derived.

behavior for bacon (2), ground beef (6), beef steak, chicken parts, pork loin (13), and minced haddock (14). With the exception of bacon and ham, none of these other foods contained curing salt inhibitors.

Apparently, the conventional method of estimating microbial resistance in irradiated pack studies has an inherent flaw. If the *D* value changes with dosage in an inoculated pack containing either a cured or an uncured product, how does one derive an MRD, particularly when a pack reflects a normal rather than an exponential distribution, as in the case of ham (3)?

The Schmidt-Nank calculation poses an additional difficulty. The more numerous the number of partial spoilage points occurring with a test organism in an inoculated pack, the greater was the difference between the minimum-maximum computed resistance values (*D*, MRD) for the organism (Tables 2, 5, 6). Moreover, the spread of values between organisms was nonuniform; hence, their relative resistances could not be compared (Tables 5, 6). Shall the minimum, or maximum,

or average value be used? An MRD maximum variation of 1.5 Mrad (strain 40B) was observed in the pork pack (Table 6), and a minimum-maximum range of as much as 3.2 Mrad (strain 62A) occurred in the ham pack (1). In the past, we had arbitrarily placed equal weights on the entire range of values and utilized the mean (1-3, 6). We are now unsure of the validity of such a practice.

The above defects are completely eliminated by the Spearman-Kärber estimation of an  $LD_{50}$ , followed by its conversion to a *D* value. As reported previously (3), this derivation of an  $LD_{50}$  is easy to apply, it is statistically sound, it readily furnishes confidence limits, and it is independent of the dose-response kinetics. The single flaw in the entire procedure lies in the transformation of the  $LD_{50}$  to a *D* value, which, as with the Schmidt-Nank technique, assumes exponential microbial death in an inoculated pack.

The versatile Weibull statistical function, however, as discussed elsewhere (3), has no such disadvantage. It can estimate any desired MRD with-

out requiring an intermediate  $LD_{50}$  or  $D$  value, and without assuming the type of death kinetics in the inoculated pack. Moreover, it can predict the probability of microbial death with any radiation dose applied, it provides information regarding the mode of death in a pack (Fig. 1), it is mathematically sound because the theoretical and graphical values are in good agreement, and it is easy to use. The only requirement for its successful employment is to design an experimental pack which will yield, ideally, a minimum of two partial spoilage "points" both above and below the 50% spoilage point. This objective was not achieved with the pork loin pack.

Table 7 emphasizes the advantage of analyzing partial spoilage data with the Weibull procedure whenever possible. Although this technique could not be used on individual strains, its application to the pooled spoilage data yielded an MRD about 18% higher than the Spearman-Kärber estimate for the viable cumulative data, and agreed closely (91%) with the highest Spearman-Kärber MRD obtained for individual (33A, 62A) strains (Table 6). Thus, when an inoculated pack study representing several strains should fail to provide calculable results for any of these organisms, pooling of the data for a Weibull approximation may save the experiment by providing significant usable data.

Of course, the most logical and direct procedure for obtaining an MRD is to use the ESD for a mutually agreed microbial population, rather than depend on a computation which may or may not interpret the correct events occurring in an inoculated pack. Since the current concept of microbiological safety requires a commercial process to reduce  $10^{12}$  *C. botulinum* spores down to  $10^0$  or its equivalent (e.g.,  $10^8$  down to  $10^{-4}$ ), this alternative method becomes extremely difficult, if not impossible, to apply, regardless of the combination of spore concentration and replicate cans; i.e., a large inoculum level with a small number of replicate cans, or a small inoculum level with a large number of replicate cans. In the first instance, it would be too difficult to prepare the spore load required, and even if this were possible, an excessively high inoculum has been found to be toxic (5, 8). In the second case, far too many replicate cans would be required to make the experiment manageable.

Based upon the above experimental results and their evaluation, we would suggest that (i) due to the incongruous behavior of the Schmidt-Nank  $D$  values with radiation dose, and the fluctuating

minimum-maximum range of  $D$  values between organisms, the latter computation should be superseded by the Weibull assay, whenever possible, or by the Spearman-Kärber estimate, in that order; and (ii) in accordance with the conventional concept of  $12D$  safety, the computed MRD of a prototype commercial radiation process for pork loin should be Weibull's value of 4.74 Mrad (Table 7).

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