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Estimation of Radiation Resistance Values of Microorganisms in Food Products

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Several statistical methods, including the conventional technique of Schmidt and Nank, were evaluated for estimating radiation resistance values of various strains of *Clostridium botulinum* by the use of partial spoilage data from an inoculated ham pack study. Procedures based on quantal response were preferred. The tedious but rigorous probit maximum likelihood determination was used as a standard of comparison. Weibull's graphical treatment was the method of choice because it is simple to utilize, it is mathematically sound, and its LD_{50} values agreed closely with the reference standard. In addition, it offers a means for analyzing the type of microbial death kinetics that occur in the pack (exponential, normal, log normal, or mixed distributions), and it predicts the probability of microbial death with any radiation dose used, as well as the dose needed to destroy any given number of organisms, without the need to assume the death pattern of the partial spoilage data. The Weibull analysis indicated a normal type kinetics of death for *C. botulinum* spores in irradiated cured ham rather than an exponential order of death, as assumed by the Schmidt-Nank formula. The Weibull 12*D* equivalent of a radiation process, or the minimal radiation dose (MRD), for cured ham was consistently higher than both the experimental sterilizing dose (ESD) and the Schmidt-Nank average MRD. The latter calculation was lower than the ESD in three of the five instances examined, which seems unrealistic. The Spearman-Kärber estimate was favored as the arithmetic technique on the bases of ease of computation, close agreement with the reference method, and providing confidence limits for the LD_{50} values.

To establish the minimal radiation dose (MRD) requirement for a given food prototype, one requires a reliable mathematical method for treating the partial spoilage data derived from an inoculated pack study. The direct spoilage data establish the minimal experimental sterilizing dose (ESD) and, as suggested by Schmidt (21), the derived 12*D* dose for the most radioresistant strain of *Clostridium botulinum* spores tested in the food involved provides the MRD. A sound experimental pack should provide the ESD. Proper mathematical treatment of the partial spoilage data, obtained from the pack, would be needed to estimate a reasonably accurate *D* value from which one calculates the 12*D*. Schmidt and Nank (22) offered the following equation for the computation of radiation *D* values from partial spoilage data:

$$D = \frac{\text{radiation dose (Mrad)}}{\log M - \log S} \qquad (1)$$

where *D* is the dose which destroys 90% of the total inoculum, assuming approximate exponential death; *M* is the total inoculum (organisms per sample units times number of units); and *S* is the number of spoiled sample units, assuming one survivor per spoiled unit.

In the course of developing prototype radiation food processes, equation 1 was used to compute *D* values. Table 1 summarizes representative partial spoilage data from an inoculated ham pack study (2), together with the respective *D* values. In every instance, the *D* values increased with increasing doses. Similar results were observed when the equation was applied to radiation partial spoilage data obtained with bacon (3), ground beef (7), beef steak, chicken parts, pork loin (22), and minced haddock (23). The *D* values reported by Schmidt and Nank (22) and by Segner and Schmidt (23) admittedly rose only slightly with increasing doses. Had their dose increments been larger, their *D* values might have increased to a greater degree.

By definition, *D* values should remain constant regardless of dose levels. The anomaly encountered above may be due either to an erroneous

TABLE 1. *Radiation resistance of representative strains of Clostridium botulinum spores in cured ham*

Strain	Radiation dose (Mrad)	Kinds of spoilage[a]			
		Swollen or toxic		With viable *C. botulinum*	
		No. of cans	*D* value[b]	No. of cans	*D* value
33A	0.5	20/20		20/20	
	1.0	19/20	0.149	17/20	0.148
	1.5	14/20	0.219	15/20	0.220
	2.0	4/20	0.271	8/20	0.282
	2.5	0/100		1/100	0.288
	3.0			0/100	
Avg			0.213		0.235
77A	0.5	20/20		20/20	
	1.0	17/20	0.168	16/20	0.167
	1.5	11/20	0.245	11/20	0.245
	2.0	0/20		5/20	0.309
	2.5			0/100	
Avg			0.207		0.240
12885A	0.5	20/20		20/20	
	1.0	18/20	0.149	19/20	0.149
	1.5	3/20	0.200	5/20	0.206
	2.0	0/20		3/20	0.267
	2.5			0/20	
	3.0			1/100	0.346
	3.5			0/100	
Avg			0.175		0.242
41B	0.5	18/20	0.072	18/20	0.072
	1.0	12/20	0.141	13/20	0.142
	1.5	6/20	0.203	6/20	0.203
	2.0	0/20		0/20	
	2.5			1/100	0.282
	3.0			0/100	
Avg			0.139		0.175
53B	0.5	20/20		19/20	0.071
	1.0	18/20	0.142	14/20	0.140
	1.5	6/20	0.199	8/20	0.203
	2.0	1/20	0.241	1/20	0.241
	2.5	0/100		0/100	
Avg			0.194		0.164

[a] Number of cans spoiled per number of cans tested.

[b] D = Mrad/(Log M−Log S)

assumption that radiation death of the test organisms in an inoculated pack is approximately exponential, in which case equation 1 does not represent the actual death kinetics, or to an erroneous supposition that each spoiled sample unit corresponds to one survivor, or it may be due to both. The mode of microbial death in an irradiated inoculated food pack has not yet been completely elucidated, and it may vary with the type of food used. However, it seems obvious that irradiated samples will contain larger numbers of surviving spores at lower doses, and will decrease progressively to one spore as the doses approach lethality. Perhaps, then, a technique is needed to estimate the most probable number (MPN) of survivors in the spoiled cans at each dose.

The concentration of spores that survive a given dosage in a replicate set of samples may be estimated by applying the equation of Halvorson and Ziegler (9):

$$\bar{x} = \frac{2.303}{a} \log \frac{n}{q}$$

where $\bar{x}$ is the MPN of organisms surviving per replicate sample; n is the total number of replicate samples per dose; q is the number of negative sample units per dose (nonswollen, nontoxic, or sterile, depending on the spoilage criterion desired); and a is the sample volume [regarded by Stumbo et al. (24) as unit volume when all sample units are of equal volume]. Hence, log S = log ($\bar{x} \times n$), and the equation 1 becomes:

$$D = \frac{\text{radiation dose (Mrad)}}{\log M - \log \left[2.303\, n \left(\log \frac{n}{q} \right) \right]} \qquad (2)$$

It is analogous to the mathematical treatment preferred by Stumbo et al. for estimating thermal D values.

As expected, both methods of computation gave practically identical D values near sterility. But as the dose decreased, with a concomitant increase in the proportion of spoiled samples, equation 2 had the advantage of producing a narrower spread of D values than equation 1 over any given range of doses. A comparison of data derived by the two calculations shows that the modified Schmidt-Nank formula (equation 2) consistently yielded somewhat higher average D values (Table 2). However, even when the number of surviving botulinal spores was estimated by a direct MPN recovery technique from irradiated preincubated ham (8), or by a direct colony count from irradiated phosphate buffer (28), D values calculated from these data increased directly with dose.

Lewis (13) pointed out that methods of computation such as those indicated above give rise to a systematic bias in the unweighted average, and lack an estimate of precision. Moreover, the determination of D values from radiation partial spoilage data presupposes that the test organism approximates exponential death under the experimental conditions imposed, which may not always be the case. For these reasons, and because the D values in various inoculated packs actually

TABLE 2. *Comparison of D values of strains of Clostridium botulinum spores, computed by various methods*

Method of computation	Type of computation	*D* values (Mrad) of strain no.				
		33A	77A	12885A	41B	53B
1. Finney (normal)	Arithmetic-graphic	0.248 ± 0.019[a]	0.254 ± 0.029	0.213 ± 0.035	0.168 ± 0.038	0.180 ± 0.020
2. Finney (log normal)	Arithmetic-graphic	0.236 ± 0.019	0.247 ± 0.022	0.200 ± 0.026	0.148 ± 0.020	0.163 ± 0.019
3. Miller-Tainter	Graphic	0.256 ± 0.040	0.250 ± 0.022	0.178 ± 0.010	0.157 ± 0.007	0.184 ± 0.021
4. Reed-Muench	Arithmetic	0.260	0.221	0.202	0.172	0.182
5. Schmidt	Graphic	0.256 ± 0.018	0.257 ± 0.029	0.222 ± 0.026	0.171 ± 0.024	0.182 ± 0.022
6. Schmidt-Nank	Arithmetic	0.235	0.240	0.242	0.175	0.164
7. Schmidt-Nank (modified)	Arithmetic	0.240	0.246	0.246	0.178	0.167
8. Spearman-Karber	Arithmetic	0.256 ± 0.023	0.257 ± 0.030	0.209 ± 0.019	0.168 ± 0.026	0.182 ± 0.021
9. Thompson	Arithmetic	0.261 ± 0.022	0.259 ± 0.024	0.206 ± 0.019	0.169 ± 0.024	0.183 ± 0.025
10. Weibull	Graphic	0.248	0.257	0.225	0.171	0.182
11. Weiss	Graphic	0.260 ± 0.016	0.250 ± 0.018	0.170 ± 0.016	0.160 ± 0.016	0.182 ± 0.016

[a] Confidence intervals computed at the 95% level.

increased with rising doses, we regard these procedures as unsatisfactory.

An inoculated pack that yields partial spoilage data is, statistically, a bioassay producing a quantal response. The test results are recorded as numbers of samples surviving (+) or sterile (−), and the numbers vary with dose. The quantal response is represented by the LD_{50} value. Schmidt (20) has shown how the LD_{50} is related to the *D* value, (from which a 12*D* process is computed) as follows:

$$D = \frac{LD_{50}}{\log A - \log 0.69} \qquad (3)$$

where LD_{50} is the dose at which 50% of the sample units are negative (nonswollen, nontoxic, or sterile, depending on the spoilage criterion desired); *A* is the initial number of organisms per sample unit; and 0.69 is the number of surviving organisms per sample unit when 50% of the sample units are negative (derived from the previously indicated equation for $\bar{x}$).

Numerous graphical and arithmetic methods, of varying degrees of complexity and accuracy, are available for estimating LD_{50} values (5, 14, 15). Only a few of these are examined here, and the viable data in Table 1 are used for computations. Among these procedures, probably the one that yields the highest possible accuracy, although extremely laborious, is the rigorous probit maximum likelihood method described in detail by Finney (6). Since it is not clear what form of distribution the partial spoilage data follow, both normal and log normal calculations were made by this method and are used here as reference standards for comparison with other determinations (Table 2).

There are many simplifications of the maximum likelihood method that produce varying losses of accuracy. Two of the simplest to apply are described by Weiss (27) and by Miller and Tainter (16). Miller and Tainter have developed an excellent graph paper that eliminates most of the arithmetic. Both of these procedures were used to derive *D* values (Table 2).

Schmidt (20) developed a method which Lewis (13) found superior to those represented by equations 1 and 2 because it gives less undue weight to results at the extremes of the dose-response range. *D* values calculated by his procedure are included in Table 2.

A simple technique for handling quantal data, which interpolates between successive responses that straddle $P = 0.5$, is the method of Reed and Muench (19). Because it is widely accepted, despite criticism by Thompson (25), it was used to derive *D* values for comparison with the rigorous probit method (Table 2). Thompson (25) objected also to Kärber's method as being a degenerate form of his own moving average interpolation, which he regards as a statistically basic treatment; it does not assume the form of the dose-response curve and it uses proper principals of graduations and interpolation. This technique, too, was used to estimate *D* values, which are presented in Table 2.

Although Thompson (25) criticized Kärber's estimate, the Spearman-Kärber assay was considered by Lewis (13) and Bross (5) to be statistically sound and preferable to other types of calculations, even for small (20 or less) sample bioassays. The Spearman-Kärber method is de-

fined as follows:

$$t_m = t_u + \frac{d}{2} - d\sum_{i=1}^{u} P_i \qquad (4)$$

where t_m is LD_{50}; t_u is the highest sublethal dose (for swelling, toxicity, or sterility, depending on the spoilage criterion desired); d is the dose increment used; u is the number of dose levels below the minimal lethal dose; and P_i = the percentage of negative sample units. D values derived by this treatment were included in Table 2.

A relatively recent contribution to probability statistics is the versatile Weibull distribution (1, 10–12, 18, 26), which is a three-parameter model. Its cumulative function is denoted by:

$$F(x) = 1 - e^{-[(x-\alpha/\eta)]^\beta} \qquad (5)$$

where F(x) is the probability of producing a negative sample unit (nonswollen, nontoxic, or sterile); x is the irradiation dose; α is the location parameter; η is the scale parameter; and β is the shape parameter. This equation has been found very useful in the reliability field where median life and other characteristics of data from unknown distributions are to be evaluated. Kao (12) suggested its application to bioassays to derive LD_{50} values. Moreover, Berrettoni (4) demonstrated the efficiency of the Weibull technique to analyze exponential, normal, and mixed distributions represented by empirical data. The usefulness of this method has been enhanced even more by the development of various forms of Weibull graph papers (11, 17, 18). Using the graph paper of Nelson (17), slightly modified,

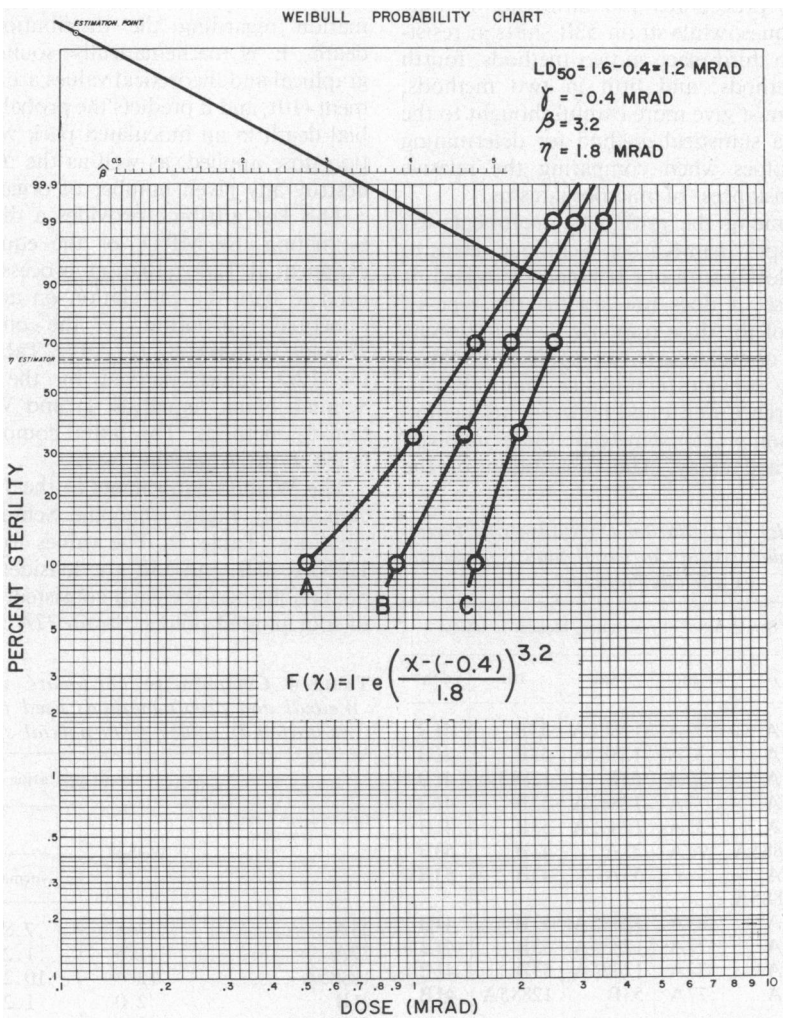

FIG. 1. *Radiation survival of Clostridium botulinum 41B spores.*

information from Table 1 was plotted to yield LD_{50} values (Fig. 1), and converted to D values (Table 2).

DISCUSSION

The comparative order of radiation resistances of the five botulinal strains, as computed by the eleven different procedures, are listed in Table 3. If rigidly applied, it can be seen that the order varied with the method of calculation. For example, strain 33A is the most resistant in four methods, second in resistance in five methods, and third in resistance in two other techniques; strain 77A is the most resistant in six procedures and second in resistance in five others. Strain 12885A varies in resistance from first place in two methods to third in seven methods, and to fourth place in two methods; strain 41B fluctuates between fourth place in two procedures and fifth place in nine other techniques, while strain 53B shifts in resistance between third place in two methods, fourth in seven methods, and fifth in two methods. Hence, one must give more careful thought to the selection of a statistical method for determining sensitivity values when comparing the relative radiation resistances of microorganisms.

Overall, among the arithmetic treatments explored, the Spearman-Kärber technique seems to agree most closely with our reference standard. It is easy to use, it does not assume the type of distribution of the dose-response data, it permits computation of even one partial spoilage "point," and it readily provides confidence limits. Practically no difference was found between this method and Thompson's (25) somewhat more involved moving average assay. The description of the procedure by Lewis (13) may be difficult to follow; hence, its application to the data in Table 1 is clarified in the Appendix.

Among the graphic procedures examined, the Weibull analysis, which is described in detail in the Appendix, is preferred for several reasons. Unlike other graphical methods, it makes no assumption regarding the type of distribution that the raw experimental data represents. In the case of the cured ham pack, it agrees most closely with the normal, rather than with the log normal probit model or with the conventional Schmidt-Nank exponential form. Chi-square tests, comparing the goodness of fit of the Weibull results with those obtained by the two probit methods, produced a closer agreement between the normal and the Weibull assay (Table 4).

The Weibull function offers additional advantages. It is simple to use, it readily provides information regarding the distribution pattern of death, it is mathematically sound because the graphical and theoretical values are in good agreement (10), and it predicts the probability of microbial death in an inoculated pack with any radiation dose applied, as well as the dose needed to destroy any given number of organisms.

This last attribute provides a direct means of estimating the MRD, or the equivalent of an exponential $12D$ radiation process, without the need to base the calculation on an assumed exponential distribution (i.e., the computation of a D value followed by $D \times 12$). Table 5 compares the $12D$ values derived by the conventional Schmidt-Nank (equation 1) and Weibull (equation 5) methods. The latter computation is detailed in the Appendix.

The Weibull equivalents to the $12D$ values are consistently higher than the Schmidt-Nank calculations (Table 5). The values for strains 33A, 12885A, 41B, and 53B are considered reasonable predictions because each consisted of a minimum of four plotted points. Strain 77A, however, pro-

TABLE 3. *Order of radiation resistance of Clostridium botulinum strains in cured ham, computed by various methods*

Method of computation[a]	Strain order of resistance, highest to lowest				
	1st	2nd	3rd	4th	5th
1.....	77A	33A	12885A	53B	41B
2.....	77A	33A	12885A	53B	41B
3.....	33A	77A	53B	12885A	41B
4.....	33A	77A	12885A	53B	41B
5.....	77A	33A	12885A	53B	41B
6.....	12885A	77A	33A	41B	53B
7.....	77A, 12885A		33A	41B	53B
8.....	77A	33A	12885A	53B	41B
9.....	33A	77A	12885A	53B	41B
10.....	77A	33A	12885A	53B	41B
11.....	33A	77A	53B	12885A	41B

[a] See sequence in Table 2.

TABLE 4. *Comparative chi-square values for the Weibull and probit methods used for estimating radiation D values from partial spoilage data*

Clostridium botulinum strain no.	Computation methods		
	Weibull	Probit	
		Normal	Log normal
33A.....	7.5	7.8	13.7
77A.....	1.6	1.2	2.7
12885A.....	12.4	10.2	5.8
41B.....	2.0	1.2	4.4
53B.....	0.9	1.0	5.1

vided only three points for plotting purposes; hence, its value is considered a questionable estimate. One should not be forced to extrapolate from three data points to obtain the three parameters of the Weibull function, although, in this instance, the plotted points straddled the 50% point.

Certain 12*D* values computed by the Schmidt-Nank formula are not supported by actual experimental evidence. For example, the ESD for strains 12885A, 41B, and 53B are higher than their respective 12*D* values. On the other hand, the Weibull equivalents are consistently higher than the ESD. This is to be expected because the ESD corresponds to the destruction of 10^8 to 10^9 spores per strain; whereas, the Weibull data estimate the destruction of 10^{12} spores per strain.

Finally, when an inoculated pack study fails to provide calculable partial spoilage data for a number of test organisms, the experiment might be saved for a Weibull analysis by combining the data and accumulating the spoilage levels among as many organisms as feasible, as shown in Table 6.

The information in Table 6, treated by the Weibull method (Fig. 2), yielded the following equation:

$$F(x) = 1 - e^{-[(x+0.5/1.95]^{3.4}}$$

Using the mean spore population per can (5.36×10^6) of the 10 botulinal strains tested, the computed 12*D* equivalent is 3.55 Mrad, which agrees with the Weibull calculations for the individual strains (Table 5).

It is realized that the estimation of *D* values by the exponential equations 1, 2, and 3 is illegitimate when partial spoilage data follow a normal distribution. At present, however, the MRD cannot be determined by any other means if the Weibull analysis is inapplicable because of inadequate data. Hence, Table 2 was prepared with this defect in mind; it serves its purpose by indicating the effect on comparative changes in resistance of the botulinal strains merely by varying the statistical handling of the data. To avoid the dilemma of making calculations from unknown modes of death kinetics, a different concept ought to be used to establish a commercially safe radiation process rather than to apply indiscriminately various statistical computations for all inoculated pack studies. Another approach to the problem will be reported at a later date.

It is hoped that this report will stimulate other investigators in this field to examine their data with other statistical treatments in addition to the popular, but less satisfactory, Schmidt-Nank method. However, regardless of the computational techniques used, an ideal inoculated pack should yield at least two partial spoilage data "points" on both sides of an LD_{50}. It is realized that this is not always possible to achieve. The various procedures described herein for calculating radiation resistance values should be applicable also for estimating equivalent resistance values from thermal process partial spoilage data.

TABLE 5. *Comparison of a 12D radiation process for cured ham computed by the Schmidt-Nank and Weibull methods from partial spoilage data*[a]

Clostridium botulinum		Experimental sterilizing dose (ESD)	Computation methods	
Strain	Spores/dose		Schmidt-Nank	Weibull
33A....	4.9×10^8	$2.5 < ESD \leq 3.0$	2.8	3.5
77A....	7.5×10^7	$2.0 < ESD \leq 2.5$	2.9	3.9[b]
12885A..	4.8×10^8	$3.0 < ESD \leq 3.5$	2.9	3.1
41B.....	7.3×10^8	$2.5 < ESD \leq 3.0$	2.1	3.5
53B.....	1.0×10^9	$2.0 < ESD \leq 2.5$	2.0	3.1

[a] From Anellis et al. (2), and Table 1.
[b] Based on three points only.

TABLE 6. *Cumulative spoilage data of irradiated cured ham inoculated with Clostridium botulinum spores*[a]

Radiation dose (Mrad)	No. of cans of ham	
	Tested	With viable *C. botulinum*[b]
0	200	183
0.5	200	171
1.0	200	151
1.5	200	64
2.0	200	21
2.5	1,000	3
3.0	1,000	2
3.5	1,000	0

[a] From Anellis et al. (2), and Table 5.
[b] The partial spoilage data from all 10 strains were combined. The mean spore population per can for the 10 strains was 5.36×10^6. $F(x) = 1 - e^{\left(\frac{x+0.5}{1.95}\right)^{3.4}}$, and $x = 3.55$ Mrad, the 12*D* equivalent.

APPENDIX

Weibull method. The viable data of strain 41B (Table 1) are used to illustrate the method. Convert the partial spoilage data, including skips, to per cent sterility (cans sterile/cans tested). Plot the per cent sterility-dose response as shown in Fig. 1 curve A. Since the plot is concave upward, make it linear by moving each point the same dose interval to the right. In curve B, each point was adjusted by a distance of

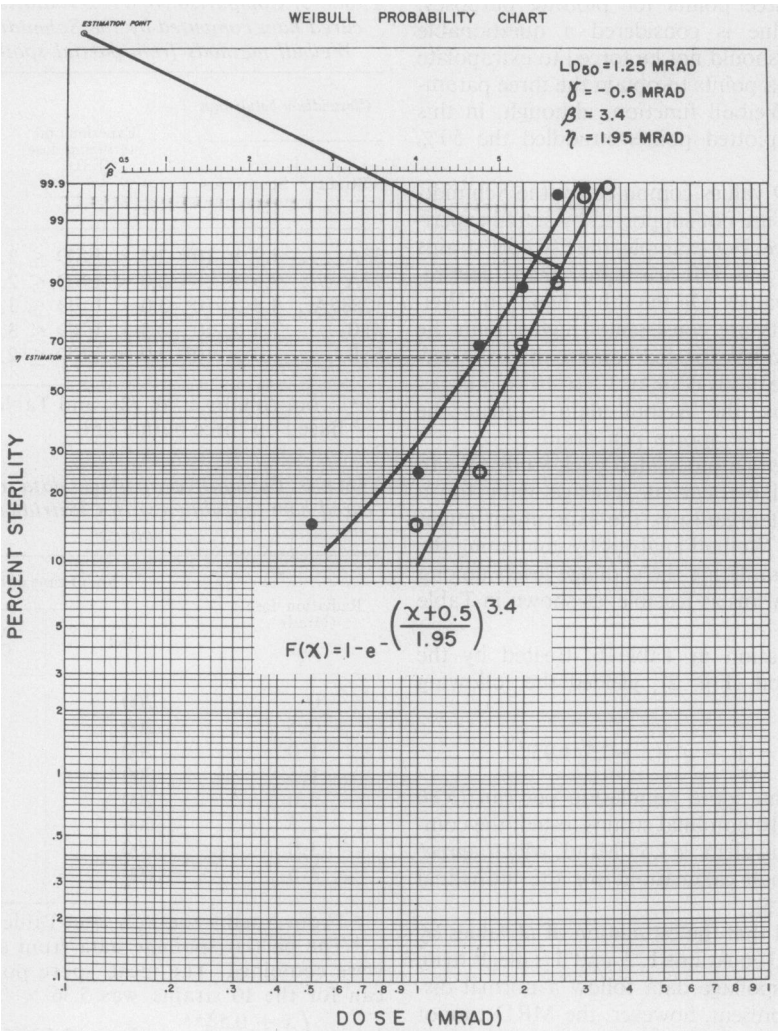

FIG. 2. *Radiation survival of Clostridium botulinum spores in cured ham. Cumulative data of all 10 strains.*

0.4 Mrad; this is the location parameter (γ). The LD_{50} is the dose (abscissa) which intersects the corrected curve at the 50% point (ordinate), or 1.6 Mrad. Since the adjustment of the curve was made to the right by adding 0.4 Mrad, correct the LD_{50} by deducting the same quantity. The corrected LD_{50} is 1.2 Mrad. Equation 3 can now be applied to compute the D value.

Normally, the adjustment for linearity is not expected to be greater than the lowest dosage plotted. If the points in curve A had been over corrected, the plot would be concave downward, as indicated by curve C. Conversely, if curve A, the initial curve, had been concave downward, it would have been necessary to move it to the left to obtain linearity, but the corrected LD_{50} would be the sum of the initial LD_{50} and the amount of adjustment to the left. Of course, if curve A is linear, no correction is necessary. Finally, if the initial data permit the fitting of a curve in either direction, it is indicative of aberrant or insufficient data (as was experienced with strain 12885A), and the results should be discarded; however, one may obtain some tentative information from such data, especially if four or more points are involved, by fitting the best straight line through these points, preferably by the least-squares technique.

Obtain the shape parameter (β) by drawing a straight line perpendicular to the corrected plot (curve B) through the estimation point in the upper left hand corner of the graph. The β value is the point where this line crosses the β scale, or 3.2. A shape parameter of approximately 1.0 indicates an exponential distribution, a value of about 2.0 denotes a log normal form, and one closer to 3.3 represents a normal distribution. The scale parameter (η), too, is read directly from the graph where curve B crosses the η estimator, or 1.8.

Substituting the above values in equation 5, we get

for strain 41B:

$$F(x) = 1 - e^{-[x-(-0.4)/1.8]^{3.2}} \quad (6)$$

This formula permits the calculation of the probability of producing sterility with any desired dose when an inoculated pack contains strain 41B. It can be used also to estimate the dose that will destroy any given number of organisms. The following example illustrates how to compute the dose equivalent to a 12*D* process, or a reduction of 10^{12} spores of strain 41B to 10^{0}. Rewrite equation 6 to:

$$1 - F(x) = e^{-(x+0.4/1.8)3.2} \quad (7)$$

where $1 - F(x)$ is the probability that a sample unit will survive.

Since 7.3×10^6 is the number of spores contained in each irradiated can, and C is the number of inoculated cans (with 7.3×10^6 spores per can) required to equal 10^{12} spores, then $C = 10^{12}/7.3 \times 10^6$, and $C = 1.37 \times 10^5$ cans. Assuming that $C + 1$ cans received a dose which sterilized all but one can, and substituting in equation 7:

$$1 - F(x) = \frac{1}{(1.37)\,(10^5) + 1} = e^{-(x+0.4/1.8)3.2} \quad (8)$$

Take the natural log (base e) of both terms:

$$-0.31481 - 5(2.30259) = -\left(\frac{x + 0.4}{1.8}\right)^{3.2}$$

Take the common log (base 10) of both sides:

$$1.0730 = 3.2 \log \frac{x + 0.4}{1.8}$$

and $x = 3.50$ Mrad.

Spearman-Kärber method. Again utilizing strain 41B, convert the partial spoilage data to per cent sterility (as in the Weibull technique). Accumulate these, between 0% and 100%, thus:

$$\sum_{i=1}^{u} P_i = 0.10 + 0.35 + 0.70 + 1.00 + 0.99 = 3.14$$

and substituting in equation 4, we get:

$$t_m = 2.5 + \frac{0.5}{2} - 0.5(3.14) = 1.18 \text{ Mrad}$$

To compute the standard error of the LD_{50} (t_m), use the equation:

$$s_m = d \sqrt{\sum_{i=1}^{u} \frac{P_i(1 - P_i)}{n_i}} \quad (9)$$

where s_m is the standard error; d and P_i are as in equation 4; and n_i is the number of samples per dose. Substituting the spoilage data, we get:

$$s_m = 0.5 \sqrt{\frac{(0.10)(0.9) + (0.35)(0.65) + (0.70)(0.30)}{20} + \frac{(0.99)(0.01)}{100}}$$

$$= 0.5 \sqrt{0.026474} = 0.0814$$

Compute the lower and upper limits for the LD_{50} at the 95% confidence interval with the following equation:

$$LD_{50} \pm 1.96\, s_m \quad (10)$$

or lower LD_{50} limit $= 1.18 - 1.96 \times 0.0814 = 1.02$, and upper LD_{50} limit $= 1.18 + 1.96 \times 0.0814 = 1.34$

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