APPLIED MICROBIOLOGY, Jan., 1967, p. 166-177
Vol. 15, No. 1

Radiation Sterilization of Prototype Military Foods

II. Cured Ham

ABE ANELLIS, D. BERKOWITZ, C. JARBOE, AND H. M. EL-BISI
Microbiology Branch, Food Division, U.S. Army Natick Laboratories, Natick, Massachusetts

Received for publication 24 August 1966

ABSTRACT

Ten lots of diced cured ham, 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). All cans were irradiated to various dose levels with Co^{60}. Evidence provided by swelling, toxicity, and recoverable *C. botulinum* with 6,350 cans demonstrated that: (i) 4.5 Mrad was more than adequate as a sterilization dose; (ii) the minimal experimental sterilizing dose (ESD) based on nonswollen nontoxic endpoints was $2.0 < \text{ESD} \leq 2.5$ Mrad, and based on nonspoiled sterile cans was $3.0 < \text{ESD} \leq 3.5$ Mrad (the latter was supported by the computed theoretical 12*D* dose); (iii) *D* values calculated from botulinal survival data indicated that, as a group, the type A strains were more radioresistant than type B strains; strains 12885A and 41B, with respective *D* values of 0.242 and 0.175, represented the most resistant of each type; (iv) swollen cans did not always contain toxin, nor were toxic cans always swollen; (v) viable *C. botulinum* can exist for 6 months at 30 C without producing visible or toxic spoilage at doses of 3.0 Mrad and lower, including, in some instances, 0.0 Mrad; and (vi) a phenomenon similar to heat activation of spores occurred at sublethal radiation doses.

As was reported elsewhere (3), the U.S. Army is engaged in a research and development program designed to determine the effectiveness of ionizing radiation for the preservation of foods and to establish guidelines for prototype radioprocesses which can be adapted for commercial production. Shelf stable bacon was the first food to be processed successfully by ionizing energy (3). Because cured ham offers a microbiologically hostile environment analogous, in certain respects, to that of bacon, it appeared logical to continue with the development of a prototype radioprocess for ham. Since the initiation of the irradiation food preservation program, 1,555 samples of hams preserved by radiation have been examined for botulinal toxin prior to evaluation by consumer taste panels. The samples, packaged either in various can sizes up to no. 10, or in flexible pouches, had received doses ranging from 1.0 to 6.0 Mrad. None of these samples was toxic to mice (Table 1).

A comprehensive study was conducted in 1964 by Osheroff, Slocum, and Decker (32) of outbreaks of botulism in the USA caused by commercial foods for the period 1906–1963. The only case of botulism traced to ham in this 57-year period occurred in 1920, 6 years before canned cured ham was introduced to the public (21). A careful examination of the Morbidity and Mortality Weekly Reports for 1964 and 1965 failed to incriminate ham in botulinal outbreaks in the USA during these 2 years. Commercial ham and ham products, therefore, have enjoyed a botulism-free record for the past 45 years. Nevertheless, it must be assumed that cured ham can be contaminated with spores of *Clostridium botulinum*, and that a successful commercial radioprocess must be capable of destroying this organism in higher numbers than are normally found in the product.

MATERIALS AND METHODS

Test organisms. Ten strains of *C. botulinum* were used: 33A, 36A, 62A, 77A, 12885A, 9B, 40B, 41B, 51B, 53B. These strains represent the highest, lowest, and intermediate radioresistances of 102 strains screened in a model system (4). The sources of these organisms, their serotypes, maintenance, and spore preparations were previously described (4). The only modifications employed were the substitution of distilled water for buffer as the spore diluent, and the use of pork-pea-agar (2) in conjunction with screw-cap

TABLE 1. *Microbiological safety of uninoculated irradiated ham*

Year examined	Irradiation dose (Mrad)	No. of containers	
		Irradiated	Free from botulinal toxin
1959	4.8	1	1
	4.5	6	6
1960	4.5	125	125
	3.0	1	1
1961	4.5	167	167
	3.0	1	1
	Unknown	12	12
1962	4.5	88	88
	2.0	1	1
	1.0	9	9
1963	4.5	84	84
	2.5	117	117
	Unknown	1	1
1964	4.5	230	230
	2.5	205	205
	1.5	16	16
	1.0	78	78
1965	6.0	4	4
	4.5	86	86
	4.0	30	30
	3.5	2	2
	3.0	47	47
	2.5	176	176
	2.0	2	2
	1.5	14	14
	1.0	52	52

tubes (13 by 254 mm) for enumerating the spore suspensions.

Food preparations. The hams used complied with military specification MIL-H-35094, 2 Nov. 1962, under the following description: cooked, chilled, boneless, cured, smoked, Type I, Class I, with a curing salt content as indicated in Table 2. The hams were cut into 1⅜-inch (3.5-cm) slices; each slice was cut into six equal "pie-shaped" wedges, and then was weighed in quantities of 100 ± 10 g into 300 × 200 C-enamel metal cans and loosely closed with lids, resulting in a ¼-inch (0.6-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.

Inoculation. The filled cans were chilled to 2 to 5 C and were inoculated by automatic syringe (Filametic Vial Filler, model A B, National Instrument Co., Inc., Baltimore, Md.) with 1.0 ml of a heat-shocked (80 C for 10 min) and chilled (1 to 2 C) spore suspension. The suspension, which was spread uniformly over the surfaces of the meat pieces, traveled freely through the crevices of the meat and open spaces in the cans. Each lot consisted of 605 cans seeded with each of the ten strains; the inoculum levels used are shown in Table 3. The cans were vacuum-sealed at 25 inches of mercury and were placed in a 3 to 5 C room overnight before irradiation. Temperature monitoring throughout this handling period indicated that the can contents never exceeded 5 C. Including appropriate controls, a total of 6,350 cans of ham were involved in this study.

Irradiation. Irradiation was performed with Co^{60} at the U.S. Army Natick Laboratories. Twenty replicate cans received dose levels of 0.5 to 2.0 Mrad in increments of 0.5 Mrad and with a dose variation of ±3%; 100 replicate cans were irradiated in the range 2.5 to 4.5 Mrad, again in steps of 0.5 Mrad, and with a dose variation of ±7%. Identical geometric configurations were used in the source for each lot of replicate cans. The radiation temperature was con-

TABLE 2. *Chemical analysis[a] of cured ham*

Ingredient	0.0 Mrad		4.5 Mrad			
			Not incubated		Incubated 6 months at 30 C	
	Range[b]	Median	Range	Median	Range	Median
$NaNO_3$, ppm	222 –309	279	128–268	180	70–189	91
$NaNO_2$, ppm	1.7 – 6.0	3.15	2.0–6.9	4.15	0.6–3.8	1.14
NaCl, %	1.27– 1.89	1.48				
Water, %	62 – 68	64.1				
Brine,[c] %	2.01– 2.71	2.26				

[a] According to the Official Methods of Analysis, Association of Official Agriculture Chemists, 1965.

[b] Uninoculated cans. Duplicate determinations were made on 12 individual samples of ham. Each sample consisted of the entire pooled contents of five replicates 300 × 200 randomly selected uninoculated sealed cans of ham; hence, a total of 60 cans were analyzed.

[c] Per cent brine $= \frac{\text{per cent NaCl}}{\text{per cent NaCl} + \text{per cent water}} \times 100.$

TABLE 3. *Inoculum levels of Clostridium botulinum spores in cured ham*

Strain no.	Radiation dose (Mrad)	Spores per can	No. of cans per dose	Total spore inoculum/strain[a]
33A	0.0–2.0[b]	4.9×10^6	20	9.8×10^7
	2.5–4.5		100	4.9×10^8
36A	0.0–2.0	1.6×10^5	20	3.2×10^6
	2.5–4.5		100	1.6×10^7
62A	0.0–2.0	6.9×10^6	20	1.4×10^8
	2.5–4.5		100	6.9×10^8
77A	0.0–2.0	7.5×10^5	20	1.5×10^7
	2.5–4.5		100	7.5×10^7
12885A	0.0–2.0	4.8×10^6	20	9.6×10^7
	2.5–4.5		100	4.8×10^8
9B	0.0–2.0	6.3×10^6	20	1.3×10^8
	2.5–4.5		100	6.3×10^8
40B	0.0–2.0	4.4×10^6	20	8.8×10^7
	2.5–4.5		100	4.4×10^8
41B	0.0–2.0	7.3×10^6	20	1.5×10^8
	2.5–4.5		100	7.3×10^8
51B	0.0–2.0	8.1×10^6	20	1.6×10^8
	2.5–4.5		100	8.1×10^8
53B	0.0–2.0	$1.0 \times .0^7$	20	2.0×10^8
	2.5–4.5		100	1.0×10^9

[a] Accumulated spore inoculum for 10 strains per dose: 20-can lots = 1.08×10^9; 100-can lots = 5.36×10^9.

[b] Doses increase in 0.5-Mrad increments.

trolled with a liquid nitrogen device, so that the centers of the can contents, which were 2 to 5 C at the beginning of irradiation, were not permitted to rise above 24 C. Ferrous sulfate dosimetry was conducted by the Radiation Sources personnel.

Assay for ham spoilage. Irradiated cans of ham were incubated at 30 C for 6 months. They were examined for swelling at weekly intervals during the first month and monthly thereafter. At the end of the incubation period, all cans were assayed for toxic spoilage and for viable *C. botulinum*.

The entire contents of each can were aseptically transferred to sterile Waring Blendor jars, diluted 1:5 (w/v) with sterile distilled water, and blended for 3 min. Samples of homogenate were centrifuged at 2,000 rev/min for 30 min, and one Swiss-Webster white mouse (15 to 20 g) per sample was injected intraperitoneally with 0.5 ml of supernatant fluid. Every sample producing symptoms of mouse intoxication within 4 days of injection was retested on two unprotected mice; two mice protected with 0.5 ml of botulinal antitoxin type A, two with antitoxin type B, and two unprotected mice received 0.5 ml of supernatant fluid which had been boiled for 10 min.

To detect the presence of viable *C. botulinum*, 5.0 ml of homogenate was inoculated into 50 ml of air-exhausted Wynne's medium (51) contained in a 60-ml screw-cap bottle. The medium was modified by reducing the agar content to 0.1% and by incorporating an additional 0.5% of glucose. An identical bottle was inoculated with a 5.0-ml sample which had been heated at 80 C for 10 min. All inoculated bottles were incubated at 30 C for 1 month. The appearance of growth within this period was confirmed for *C. botulinum* with the mouse toxicity test described above.

Calculation of radioresistance. The equation of Schmidt and Nank (43) was used to compute the

TABLE 4. *Effect of Co^{60} irradiation on spoilage of cured ham inoculated with Clostridium botulinum spores*

Strain no.	Radiation dose (Mrad)	No. of cans of ham			
		Tested	Swollen	With botulinal toxin	With viable *C. botulinum*
33A	0.0	20	19	16	20
	0.5	20	20	20	20
	1.0	20	19	19	17
	1.5	20	14	14	15
	2.0	20	4	4	8
	2.5	100	0	0	1
	3.0–4.5[a]	100	0	0	0
36A	0.0	20	18	9	20
	0.5	20	17	17	18
	1.0	20	18	18	19
	1.5	20	0	0	4
	2.0	20	0	0	3
	2.5–4.5	100	0	0	0
62A	0.0	20	19	11	16
	0.5	20	14	15	18
	1.0	20	20	20	20
	1.5	20	0	1	4
	2.0	20	0	0	1
	2.5	100	0	0	0
	3.0	100	0	0	1
	3.5–4.5	100	0	0	0
77A	0.0	20	17	9	20
	0.5	20	20	20	20
	1.0	20	17	17	16
	1.5	20	11	11	11
	2.0	20	0	0	5
	2.5–4.5	100	0	0	0
12885A	0.0	20	18	12	19
	0.5	20	20	20	20
	1.0	20	18	18	19
	1.5	20	3	3	5
	2.0	20	0	0	3
	2.5	100	0	0	0
	3.0	100	0	0	1
	3.5–4.5	100	0	0	0
9B	0.0	20	20	15	20
	0.5	20	20	18	20
	1.0	20	12	12	19
	1.5	20	0	0	3
	2.0	20	0	0	0
	2.5–4.5	100	0	0	0

TABLE 4—*Continued*

Strain no.	Radiation dose (Mrad)	No. of cans of ham			
		Tested	Swollen	With botulinal toxin	With viable *C. botulinum*
40B	0.0	20	18	14	20
	0.5	20	20	20	12
	1.0	20	9	20	10
	1.5	20	0	1	7
	2.0	20	0	0	0
	2.5–4.5	100	0	0	0
41B	0.0	20	20	19	20
	0.5	20	18	18	18
	1.0	20	12	12	13
	1.5	20	6	6	6
	2.0	20	0	0	0
	2.5	100	0	0	1
	3.0–4.5	100	0	0	0
51B	0.0	20	19	7	8
	0.5	20	3	1	6
	1.0	20	0	0	4
	1.5	20	0	0	1
	2.0	20	0	0	0
	2.5	100	0	0	1
	3.0–4.5	100	0	0	0
53B	0.0	20	20	16	20
	0.5	20	20	20	19
	1.0	20	18	18	14
	1.5	20	6	6	8
	2.0	20	1	1	1
	2.5–4.5	100	0	0	0

[a] Doses increase in 0.5-Mrad increments.

radiation D values (decimal reduction doses) of the 10 test organisms. Partial spoilage data as well as recovery of *C. botulinum* from nonspoiled cans were employed in these computations. It is currently assumed, as propounded by Schmidt (42), that a microbiologically safe radioprocess should conform with a 12-log spore reduction of *C. botulinum*, ostensibly analogous to the practices followed by the canning industry for commercial thermally processed foods. Hence, sterilizing doses for each strain were calculated by the formula $D \times 12$.

RESULTS

Minimal sterilization dose. Table 4 details the spoilage data for each of the 10 strains at all radiation levels, and Table 5 totals the data among the ten strains. Lots of 1,000 cans per dose, containing an accumulated spore population of 5.4×10^9 (Table 3), and challenged by 3.5 Mrad or higher, were found unswollen, nontoxic, and sterile. Similar lots, irradiated to 3.0 or 2.5 Mrad, were entirely free from swelling and toxic spoilage. However, of 1,000 cans exposed to 3.0 Mrad, only two cans contained inert but recoverable spores (strains 62A and 12885A), and, among the 1,000 cans subjected to 2.5 Mrad, just three cans harbored dormant *C. botulinum* (strains 33A, 41B and 51B).

TABLE 5. *Cumulative spoilage data of irradiated cured ham inoculated with Clostridium botulinum spores*

Radiation dose (Mrad)	Total spore population (pooled 10 strains)	No. of cans of ham			
		Tested	Swollen[a]	With botulinal toxin[b]	With viable *C. botulinum*[c]
0	0	100	74	0	0
0	1.1×10^9	200	188	128	183
0.5	1.1×10^9	200	172	169	171
1.0	1.1×10^9	200	143	154	151
1.5	1.1×10^9	200	40	42	64
2.0	1.1×10^9	200	5	5	21
2.5	5.4×10^9	1,000	0	0	3
3.0	5.4×10^9	1,000	0	0	2
3.5	5.4×10^9	1,000	0	0	0
4.0	5.4×10^9	1,000	0	0	0
4.5	5.4×10^9	1,000	0	0	0

[a] $D = 0.165$; $12D = 1.98$.
[b] $D = 0.165$; $12D = 1.98$.
[c] $D = 0.212$; $12D = 2.54$.

The 200-can lot of ham, which had a total spore load of 1.1×10^9 and received 2.0 Mrad, contained only five swollen toxic cans, and had viable botulinal organisms. An additional 16 cans were unswollen and nontoxic, but had dormant recoverable *C. botulinum*. Doses below 2.0 Mrad gave increasing amounts of spoilage with decreasing radiation doses.

Unirradiated inoculated controls did not manifest 100% visible or toxic spoilage; furthermore, some of the 200 cans actually became sterile after 6 months of incubation (Table 5). The overall spoilage pattern was 94% swollen and 64% toxic cans, and 91.5% had recoverable *C. botulinum*. Apparently strain 51B spores were the least capable of producing spoilage in the ham environment, yet they produced a relatively long surviving "tail" with increasing dosage (Table 4). Of the 100 unirradiated uninoculated controls, 74% visibly spoiled, but no toxin or viable *C. botulinum* could be found. The spoilage, of course, was caused by indigenous nonbotulinal organisms which, in many instances (26%), were inhibited by the curing salts.

The minimal experimental sterilizing dose (ESD), as based on the three criteria of spoilage, are indicated in Table 6. Using both visible and toxic spoilage, sterility was obtained with 2.0

TABLE 6. *Experimental sterilizing dose (ESD) based on different criteria of spoilage by Clostridium botulinium in irradiated cured ham*

Strain no.	Minimal radiation sterilization dose (Mrad) to eliminate		
	Swelling	Botulinal toxin	Viable *C. botulinum*
33A	$2.0 < \text{ESD} \leq 2.5$	$2.0 < \text{ESD} \leq 2.5$	$2.5 < \text{ESD} \leq 3.0$
36A	$1.0 < \text{ESD} \leq 1.5$	$1.0 < \text{ESD} \leq 1.5$	$2.0 < \text{ESD} \leq 2.5$
62A	$1.0 < \text{ESD} \leq 1.5$	$1.5 < \text{ESD} \leq 2.0$	$3.0 < \text{ESD} \leq 3.5$
77A	$1.5 < \text{ESD} \leq 2.0$	$1.5 < \text{ESD} \leq 2.0$	$2.0 < \text{ESD} \leq 2.5$
12885A	$1.5 < \text{ESD} \leq 2.0$	$1.5 < \text{ESD} \leq 2.0$	$3.0 < \text{ESD} \leq 3.5$
9B	$1.0 < \text{ESD} \leq 1.5$	$1.0 < \text{ESD} \leq 1.5$	$1.5 < \text{ESD} \leq 2.0$
40B	$1.0 < \text{ESD} \leq 1.5$	$1.5 < \text{ESD} \leq 2.0$	$1.5 < \text{ESD} \leq 2.0$
41B	$1.5 < \text{ESD} \leq 2.0$	$1.5 < \text{ESD} \leq 2.0$	$2.5 < \text{ESD} \leq 3.0$
51B	$0.5 < \text{ESD} \leq 1.0$	$0.5 < \text{ESD} \leq 1.0$	$2.5 < \text{ESD} \leq 3.0$
53B	$2.0 < \text{ESD} \leq 2.5$	$2.0 < \text{ESD} \leq 2.5$	$2.0 < \text{ESD} \leq 2.5$

TABLE 7. *Relative radioresistance of Clostridium botulinum in cured ham*

Strain no.	Avg radiation *D* values based on		
	Swelling	Botulinal toxin	Viable *C. botulinum*
33A	0.213	0.213	0.235
36A	0.143	0.143	0.218
62A	0.071	0.072	0.214
77A	0.207	0.207	0.240
12885A	0.175	0.175	0.242
9B	0.142	0.108	0.171
40B	0.143	0.189	0.143
41B	0.139	0.139	0.175
51B	0.065	0.061	0.166
53B	0.194	0.194	0.164

$< \text{ESD} \leq 2.5$ Mrad with the inactivation of strains 33A, and 53B; but to attain nonspoiled ham free from *C. botulinum* required the inactivation of strains 62A and 12885A with $3.0 < \text{ESD} \leq 3.5$ Mrad.

Radioresistance of C. botulinum. Information in Table 4 was used to compute *D* values for the three types of spoilage produced by each botulinal strain. In general, visible and toxic spoilage resulted in similar *D* values, and viable data gave the highest *D* values. Table 7 lists the average relative radioresistances of the 10 test strains. Based on survival *D* values, the type A strains as a group appeared to be of higher radioresistance than the type B group. Strains 12885A and 77A were the most resistant, followed closely by 33A, whereas 36A and 62A were of lower but of comparable resistance. Among the type B strains, the least sensitive organisms were 41B and 9B, followed by 51B and 53B, which were of identical resistance, with 40B of least tolerance.

In two cases (41B and 53B), visible spoilage *D* values were higher than recovery *D* values, and in three situations (40B, 41B, and 53B) toxic spoilage *D* values were higher than survival *D* values. Theoretical 12*D* estimates generally agreed with the ESD values (Table 8). The highest 12*D* dose was 2.90 Mrad (corresponding with strain 12885A), which was somewhat below $3.0 < \text{ESD} \leq 3.5$. In comparison, the 12*D* dose for the accumulated spoilage data (Table 5) was 1.98 Mrad for both visible and toxic spoilage, and 2.54 Mrad on the basis of viable *C. botulinum*.

Effect of sublethal doses on spoilage. Irradiation of ham to 0.5 Mrad resulted in a markedly increased rate of spoilage over unirradiated controls during the first week of incubation (Fig. 1). A dose of 1.0 Mrad also caused a significant increase in spoilage over the controls but to a lesser degree than with 0.5 Mrad. Even 1.5 Mrad produced more rapid swelling in cans containing strains 33A and 77A than with the controls, but to a still lesser extent than with 1.0 Mrad. This dose-spoilage response was observed with most strains up to 1 month of incubation, after which the unirradiated samples equaled or surpassed the irradiated spoilage pattern. Ham infected with strain 51B did not show this phenomenon at any dose level.

DISCUSSION

Recently, doubts have been voiced concerning the validity of applying the 12*D* concept for the establishment of a safe prototype radioprocess (15, 17). Moreover, Dyer et al. (14) found that a radioprocess for crabmeat, based on *D* values derived from survival curves which "tailed," gave a process lower than one based on experimentally determined doses. Table 8 also indicates that the 12*D* doses of some strains (62A, 12885A, 41B, 51B, and 53B) are lower than their respective ESD values.

The method for computing the radiation *D* value can greatly affect the radioprocess. Not

TABLE 8. *Minimal radiation sterilization doses for cured ham inoculated with Clostridium botulinum spores*

Strain no.	Total spores per dose	Experimental sterilizing dose (ESD)[a]	Computed avg $12D^b$ dose based on		
			Swelling	Botulinal toxin	Viable *C. botulinum*
		Mrad	*Mrad*	*Mrad*	*Mrad*
33A	4.9×10^8	$2.5 < \text{ESD} \leq 3.0$	2.56	2.56	2.82
36A	3.2×10^6	$2.0 < \text{ESD} \leq 2.5$	1.72	2.62	2.62
62A	6.9×10^8	$3.0 < \text{ESD} \leq 3.5$	0.85	0.86	2.57
77A	7.5×10^7	$2.0 < \text{ESD} \leq 2.5$	2.48	2.48	2.88
12885A	4.8×10^7	$3.0 < \text{ESD} \leq 3.5$	2.10	2.10	2.90
9B	1.3×10^8	$1.5 < \text{ESD} \leq 2.0$	1.70	1.30	2.05
40B	8.8×10^7	$1.5 < \text{ESD} \leq 2.0$	1.72	2.27	1.72
41B	7.3×10^8	$2.5 < \text{ESD} \leq 3.0$	1.67	1.67	2.10
51B	8.1×10^8	$2.5 < \text{ESD} \leq 3.0$	0.78	0.73	1.99
53B	1.0×10^9	$2.0 < \text{ESD} \leq 2.5$	2.33	2.33	1.97

[a] Flat, nontoxic sterile cans.
[b] Decimation through 12-log cycles of initial spore load by the equation $D \times 12$.

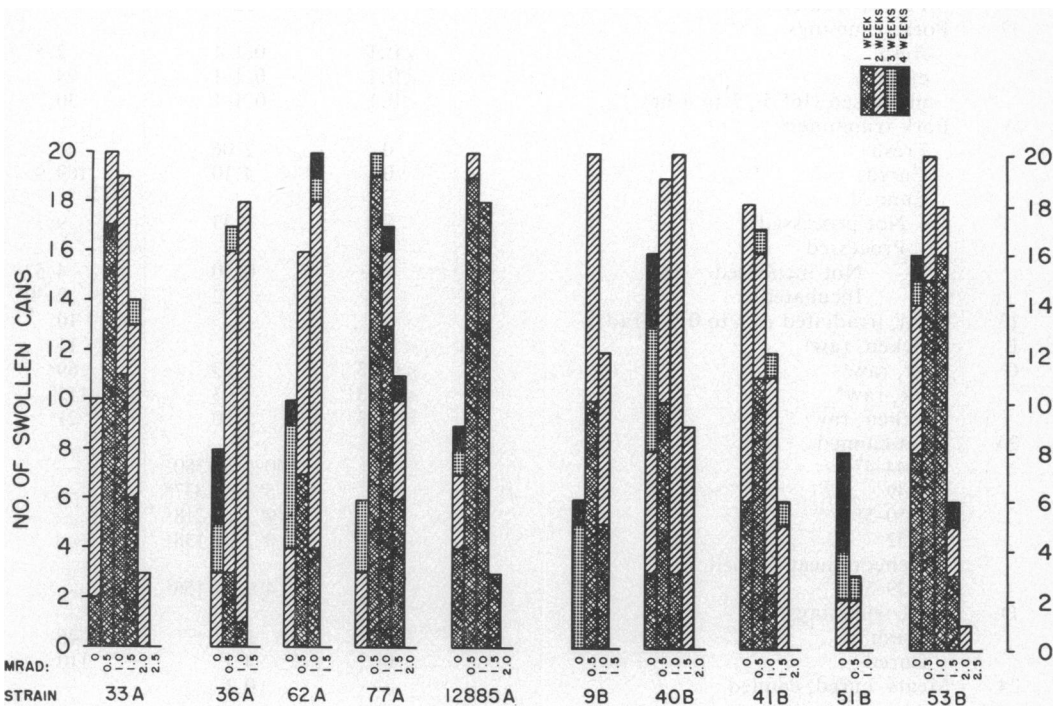

FIG. 1. *Effect of sublethal radiation doses on swelling of cans of cured ham inoculated with Clostridium botulinum. Results represent 4 weeks of incubation at 30 C. Missing time bars indicate no increase in number of swollen cans over previous time period.*

only does the computed minimal sterilizing dose depend directly upon the method of calculation, but a change in the relative radioresistances of the botulinal strains may occur (*in preparation*).

If the computed average 12*D* dose is accepted as a valid criterion for a safe prototype radioprocess, then canned cured ham can be sterilized with a dose of 2.90 Mrad (Table 8). The maximal ESD of ham challenged by a total population of 5.4×10^9 botulinal spores is somewhere between 3.0 and 3.5 Mrad (Table 6), and fully meets the rigorous safety requirement of the mandatory absence of viable *C. botulinum* in unspoiled food product. On the basis of spoilage data, both 3.0

TABLE 9. *Incidence of indigenous putrefactive anaerobic spores in meat products*

Reference[a]	Meat product	No. of spores/g of meat		
		Low	Usual	High
5	Trimmings, fresh	—	1.5	42
	Pork trimmings, fresh	<3	—	51
	Beef			
	Fresh	—	6.5	—
	Knuckle (inside and outside round)	<0.006	0.007–0.06	1.4
6	Beef, raw			
	In cartons	<0.0006	0.007–0.06	1.4
	In cans			
	Surface	<0.005	0.005–0.05	0.13
	Core	<0.01	0.012–0.07	0.12
	Beef, processed (F_0 = 0.01)	0	0	0
7	Rind pig carcass at inspection end of slaughter line	0	4	40
8	Chicken, raw, uneviscerated	<10[b]	—	<200[b]
9	Ham			
	Entire 14 lb	—	0.4	—
	Pasteurized (150 F)	—	12[c]	—
11	Pork trimmings			
	fresh	<0.1	0.1–1	2.5
	cured	<0.1	0.1–1	24
	processed (165 F, 3 to 4 hr)	<0.1	0.1–1	30
A	Pork trimmings[d]			
	Fresh	0	2.06	46
	Cured	0	4.10	109.9
	Canned			
	Not processed	0	1.33	9.3
	Processed			
	Not incubated	0	0.70	4.52
	Incubated	0	1.32	9.30
13	Ham, irradiated (0.3 to 0.5 Mrad)	—	—	10
B	Chicken, raw[d]	<1	1–2	11
C	Beef, raw[d]	<0.33	3.03	69[e]
	Pork, raw[d]	<0.33	3.03	115[f]
	Chicken, raw[g]	<0.33	2.50	21[h]
20	Meat canned			
	1944–47	—	40% of 380[i]	—
	1949	—	5% of 337[i]	—
	1950–51	—	0.9% of 218[i]	—
	1952	—	0% of 138[i]	
	Luncheon meat canned			
	1949–52	—	6.4% of 156[i]	—
D	Pork trimmings[d]			
	Fresh	0	2.1	46
	Cured	0	4.1	110
24	Meats, cured, canned	—	0.1	—

and 2.5 Mrad are sterilizing doses, with a very small fraction of the cans (0.25%) containing inert but recoverable *C. botulinum* (Table 5).

It is probable that a 3.0- or even a 2.5-Mrad commercial radioprocess would be a microbiologically safe process. It must be remembered that our experiments imposed an extremely unrealistic spore burden upon the radioprocess. Table 1 shows that 717 samples of radiation-preserved uninoculated cured hams were free from botulinal toxin with doses of 3.0 Mrad down to 1.0 Mrad, and were consumed by test panels. It must also be borne in mind that cured canned meats have never received the minimal prescribed thermal botulinal "cook" (F_0 = 2.78). Instead, they are given a sublethal F_0 of only 0.1 to 0.6 (18, 20, 24, 37) with a median value of 0.2 (20). Yet, these thermally underprocessed foods have a perfect public safety record (32). The reasons for this enviable health record are at least

TABLE 9—*Continued*

Reference[a]	Meat product	No. of spores/g of meat		
		Low	Usual	High
25	Ham, pasteurized, canned			
	Sound	<3	—	—
	Flipper	—	3–30	—
	Swollen	<3	1–10	10
26	Meats for over 20 years	<0.1	—	40
34	Pork trimmings			
	Fresh	—	<3	51
	Cured	—	<3	51
	Pork luncheon meat	<0.18	<2	—
	Beef, fresh	<0.1	6.5	—
38	Trimmings, fresh	—	1.5	42
	Pork trimmings	—	<3	51
41	Ham, raw	0.01	0.04	0.086
48	Luncheon meat, raw	—	0.63	1
50	Pork trimmings			
	Fresh	<0.18	—	51
	Cured	<0.18	—	43
	Pork luncheon meat, canned	<0.18	—	4
	Beef trimmings, fresh	<0.23	1.8	46
E	Meats	<0.3	0.99	10
F	Rind pig carcass at inspection end of slaughter line	—	2[i]	—

[a] Numbers refer to references in the Literature Cited section. Letters represent the following: (A) V. Conquest, *unpublished data*; (B) R. A. Greenberg, B. O. Bladel, and R. S. Kittaka, Bacteriol. Proc., p. 1–2, 1965; (C) R. A. Greenberg and R. B. Tompkin, *unpublished data*; (D) L. A. Harriman et al., *unpublished data*; (E) A. R. Miller, *personal communication*; (F) D. C. Wilson, *personal communication*.

[b] Total anaerobic spores.

[c] Includes clostridia, bacilli, cocci.

[d] Samples were tested for *C. botulinum* spores, but none was found.

[e] Only one sample of 624.

[f] Only one sample of 656.

[g] Samples tested for *C. botulinum*; one type C spore found.

[h] Only one sample of 1,078.

[i] Per cent positive of number of samples tested. Number of putrefactive anaerobic spores not given

[j] Total anaerobic spores. One *C. sporogenes* and one *C. perfringens* in a total of 12 factories.

twofold: (i) a very low natural level of total mesophilic clostridial spores in raw meats (5–9, 11, 13, 20, 24–26, 34, 38, 41, 48, 50) which has steadily been decreasing with improved sanitary practices over the years (20, 38), and (ii) the presence of curing salts which inhibit germination, subsequent outgrowth, or toxin production of the apparently heat-injured spores (1, 10, 11, 19, 21, 27, 29–31, 33–36, 38, 39, 44–49). [An excellent review of the technical literature on the effect of curing agents on microflora from the earliest times up to about 1952 was published by Jensen (26).]

Numerous laboratories have surveyed meats and meat products for mesophilic putrefactive anaerobic (PA) spores; some included a search for *C. botulinum*. Greenberg et al. (19*a*) recently completed an extensive 12-month survey for these organisms in raw beef, chicken, and pork in seven representative areas in the USA and Canada. They analyzed 2,358 meat samples (1 lb each) and isolated 19,727 clostridial colonies, obtaining an average of 2.8 spores per g of meat, with a range of < 0.33 to 115; 31.3% of the samples contained < 1 spore per g, and 76.8% had < 3 spores per g. Of these isolates, only one was characterized as *C. botulinum* (type C), yielding a ratio of PA spores to botulinal spores of 20,000:1.

Table 9 summarizes the findings of the known surveys on the incidence of clostridial spores in meats and meat products. The data clearly indicate that indigenous mesophilic anaerobic spores occur in very small numbers, and, among these, the presence of *C. botulinum* is a relatively rare event.

Under the conditions of the experiment, a radiosterilizing dose must inactivate 10 strains

totaling in excess of 10^9 botulinal spores (Table 5). Assuming a maximal natural PA spore contamination level of 200/g (Table 9), a 100-g can of ham could contain a maximum of 20,000 PA spores, and, hence, may harbor about one *C. botulinum* spore (19*a*), or an accumulated population of 10^3 spores per dose. The ESD, therefore, represents an excess of at least six log cycles of destruction. If interpreted in terms of the 12*D* probabilistic safety criterion (15, 17, 42), the experimental pack of 1,000 cans per dose would be equivalent to, and would qualify the safety of, at least 10^9 commercial uninoculated cans; thus, the probability hazard-level is less than 10^{-9}.

The suppressive qualities of curing salts in commercial thermally processed hams have been noted many times Some evidence exists that these ingredients also exhibit inhibitory properties for bacteria in irradiated meats. Krabbenhoft et al. (29) found that ground beef containing 10^6 *C. botulinum* spores per g of meat could not be sterilized by 3.5 Mrad of γ rays in the absence of curing compounds. The spores were destroyed, however, with 2.0 Mrad in the presence of 1,000 ppm of $NaNO_3$ and 2.5% NaCl, and at 2.5 Mrad in the presence of 200 ppm of $NaNO_2$ and 2.5% NaCl. Use of these salts individually, together with radiation, was ineffective. The studies by Anellis et al. (3, 4) indicated that the spores of several strains of *C. botulinum* (33A, 36A, 12885A, 9B, 41B, 53B) have a smaller radiation *D* value in mildly heated cured bacon (3) than in phosphate buffer, *p*H 7.0 (4). Greenberg et al. (18) determined radiation *D* values of botulinal spores in thermally processed cured ham, and concluded that resistance was "considerably below those typically found in uncured food substrates." Kempe and Graikoski (27) also reported inhibition of *C. botulinum* by the curing salts in canned pork luncheon meat, whether the inoculated meat was or was not irradiated.

There is some reason to believe that irradiated, nonsterile, cured ham may be equivalent in safety to commercial heat-processed nonsterile cured ham. For example, Hansen (22) and Hansen and Warnoe (23) investigated the combined effect of mild heat and low dose radiation on uninoculated cured ham in 1,200 cans. They applied either of two thermoprocesses: one with an $F_0 = 0.0003$ (to 75 C central ham temperature), and the second too low to have a meaningful F_0 value (to 65 C central temperature). Each of the two thermoprocesses was evaluated when applied in pre- and postcombination with Co^{60} radiation to 0.2, 0.3, and 0.6 Mrad. They reported 100% "commercial sterility" with a dose of 0.6 Mrad, and 97% with 0.3 Mrad, with either heat treatment in either order. A 0.2-Mrad dose yielded 86% sterility if the hams were preheated, and 64% if postheated to 75 C. Knudsen (28), too, successfully obtained "commercial sterility" of cured ham with doses of 0.4 to 0.5 Mrad by heating the meat to a center temperature of only 65 or 75 C.

Greenberg et al. (18) heat-processed ham infected with *C. botulinum* spores (173 and 2,670/g) to $F_0 = 0.2$, and irradiated other samples of identically inoculated ham to 0.5 to 3.5 Mrad in 1.0-Mrad increments. Their spoilage data, regardless of spore load, indicated that radiation doses above 0.5 Mrad "induce the same stability (and safety) in cured meats as do the nonsterilizing thermal processes employed for decades by industry." Riemann (37) also investigated the possibility of developing a radioprocess for cured ham which would be equivalent, microbiologically, to that of a commercial thermal process. Using spores of putrefactive anaerobe PA3679, he found that a radiation dose of 0.55 Mrad was equivalent to an $F_0 = 0.6$ in lethality. More importantly, about 10% of the spores surviving the heat treatment were able to grow out in the cured ham on prolonged incubation, whereas only 0.013% of the survivors of a 1.0-Mrad dose were capable of growth under identical conditions. He concluded "that the main difference between the results of heat processing and irradiation is that dormancy is more pronounced after the latter treatment." Hansen and Warnoe (23), too, experienced a "degree of sterility a little higher [with 0.6-Mrad radiation] than that obtained by the norman heat processings [$F_0 =$ 0.2 to 0.6] used in current practice" for cured hams.

Scott (45) suggested that neither the curing ingredients per se, nor the *p*H of the ham are inhibitory, but, rather, inhibition is produced by the water activity (a_w) produced primarily by the NaCl, which can cause bacteriostasis. He found that liquid media with $a_w = 0.95$, which apparently prevails in Australian hams, were marginal for the growth of some strains of *C. botulinum* types A and B, but were ineffective for other strains.

In our studies, cans of cured ham containing *C. botulinum* spores, irradiated with certain sublethal doses (0.5 to 1.5 Mrad), clearly evidenced an acceleration in visible spoilage over those of the unirradiated controls (Fig. 1). Cann et al. (12) observed a similar phenomenon with the rate of toxin production in vacuum-packed fish containing type E botulinal spores when irradiated with 0.3 Mrad. Although the data of Greenberg et al. with ham and curing salts (18), and of Krabbenhoft et al. with beef and curing salts (29) showed spoilage only on final rather than early storage

time, their results, too, indicated, but to a very limited extent, an enhanced susceptibility of the meats to swelling and toxin production with lower radiation doses.

The phenomenon may be attributed to one or more of the following factors: (i) a decrease in the indigenous microbial flora by radiation, thus reducing the competitive microenvironment for the germination and outgrowth of *C. botulinum* spores; (ii) a significant lowering of the nitrite-nitrate concentration by radiation, thus decreasing the bacteriological stability of the ham; and (iii) a true radiation activation of spores in specific substrates, analogous to heat activation.

Cann et al. (12) suggested that the effect might be due to the first possibility. If true, increased spoilage with certain sublethal doses could be expected in all food products. Thus far, the effect was not observed at any radiation level in chicken or pork inoculated with botulinal spores and handled in a manner identical to the present study with cured ham (*unpublished data*).

Erdman and Watts (16) reported that irradiation of ham caused a reduction of nitrate to nitrite, followed by a decrease of the nitrite content with storage time. Bulman and Ayres (10), too, observed a decrease of nitrite with time. Kempe and Graikoski (27) thought that a change in the concentration of these salts might shorten the microbiological stability of the cured meat. Results in Table 2 indicate a decrease in nitrate content when ham was irradiated to 4.5 Mrad and an additional decline after 6 months of storage at 30 C. The nitrite level may not have increased significantly with radiation to 4.5 Mrad, but it apparently was lower after the 6-month storage period. Additional randomly selected cans of ham, in replicates of 10, were irradiated in our laboratory from 0 to 4.5 Mrad in increments of 0.5 Mrad and were assayed for nitrate and nitrite concentrations without storage. The data did not show a reduction of either chemical at any dose level. The information in the literature is insufficient to indicate the effect of a decrease in nitrate-nitrite content in cured meats on the microbiological stability of ham. Further study of this factor is warranted.

Support for the possibility of a true radiation activation of spores occurring with sublethal doses was reported by Roberts and Ingram (40). They apparently observed an increase in counts of an unheated aqueous suspension of *C. bifermentans* spores when irradiated with very low doses. In the case of the accelerated botulinal spoilage of cured ham, one must not overlook the possibility of a concurrent ionic germination effect with radiation. The possibility of such an occurrence is now under investigation.

ACKNOWLEDGMENT

We gratefully acknowledge the chemical analyses of our samples by the Chemistry Branch, and the dosimetry measurements and irradiations by the Radiation Sources Branch, Food Division, U.S. Army Natick Laboratories.

LITERATURE CITED

1. AMERICAN MEAT INSTITUTE FOUNDATION. 1960. The science of meat and meat products. W. H. Freeman and Co., San Francisco.
2. ANDERSON, A. A. 1951. A rapid plate method of counting spores of Clostridium botulinum. J. Bacteriol. **62**:425–432.
3. ANELLIS, A., N. GRECZ, D. A. HUBER, D. BERKOWITZ, M. D. SCHNEIDER, AND M. SIMON. 1965. Radiation sterilization of bacon for military feeding. Appl. Microbiol. **13**:37–42.
4. ANELLIS, A., AND R. B. KOCH. 1962. Comparative resistance of strains of *Clostridium botulinum* to gamma rays. Appl. Microbiol. **10**:326–330.
5. AYRES, J. C. 1954. Microorganisms associated with the spoilage of thermal processed meats, p. 15–25. *In* Quality and stability of canned meats, a symposium sponsored by Quartermaster Food and Container Institute for the Armed Forces.
6. AYRES, J. C., AND A. T. ADAMS. 1953. Occurrence and nature of bacteria in canned beef. Food Technol. **7**:318–323.
7. BALTZER, J., AND D. C. WILSON. 1965. The occurrence of clostridia on bacon slaughter lines. J. Appl. Bacteriol. **28**:119–124.
8. BARNES, E. M., AND D. H. SHRIMPTON. 1957. Causes of greening of uneviscerated poultry carcasses during storage. J. Appl. Bacteriol. **20**: 273–285.
9. BROWN, W. L., C. A. VINTON, AND C. E. GROSS. 1960. Heat resistance and growth characteristics of microorganisms isolated from semiperishable canned hams. Food Res. **25**:345–350.
10. BULMAN, C., AND J. C. AYRES. 1952. Preservative effect of various concentrations of curing salts in comminuted pork. Food Technol. **6**:255–259.
11. BURKE, M. V., K. H. STEINKRAUS, AND J. C. AYRES. 1950. Methods for determining the incidence of putrefactive anaerobic spores in meat products. Food Technol. **4**:21–25.
12. CANN, D. C., B. B. WILSON, G. HOBBS, AND J. M. SHEWAN. 1965. The growth and toxin production of *Clostridium botulinum* type E in certain vacuum packed fish. J. Appl. Bact. **28**:431–436.
13. DRAKE, S. D., J. B. EVANS, AND C. F. NIVEN. 1960. The effect of heat and irradiation on the microflora of canned hams. Food Res. **25**:270–278.
14. DYER, J. K., A. W. ANDERSON, AND P. DUTIYABODHI. 1966. Radiation survival of food pathogens in complex media. Appl. Microbiol. **14**: 92–97.
15. EL-BISI, H. M. 1965. Principles of microbiological safety and stability of radiation sterilized foods. Natl. Acad. Sci.—Natl. Res. Council Publ. 1273, p. 223–232.

16. ERDMAN, A. M., AND B. M. WATTS. 1957. Radiation preservation of cured meats. Food Technol. **11**:349–353.
17. GOLDBLITH, S. A. 1965. Some thoughts on the microbiology and wholesomeness aspects of irradiated foods, p. 55–57. *In* Radiation processing of food. Hearings before the Joint Committee on Atomic Energy, Congress of the United States. U.S. Govt. Printing Office, Washington, D.C.
18. GREENBERG, R. A., B. BLADEL, AND W. J. ZINGLEMANN. 1965. Radiation injury of *Clostridium botulinum* spores in cured meat. Appl. Microbiol. **13**:743–748.
19. GREENBERG, R. A., J. H. SILLIKER, AND L. D. FATTA. 1959. The influence of sodium chloride on toxin production and organic breakdown in perishable cured meat inoculated with *Clostridium botulinum*. Food Technol. **13**:509–511.
19*a*. GREENBERG, R. A., R. B. TOMPKIN, B. O. BLADEL, R. S. KITTAKA, AND A. ANELLIS. 1966. Incidence of mesophilic *Clostridium* spores in raw pork, beef, and chicken in processing plants in the United States and Canada. Appl. Microbiol. **14**:789–793.
20. GROSS, C. E. 1954. Study of the incidence of spoilage organisms in canned meat products manufactured under commercial conditions, p. 26–36. *In* Quality and stability of canned meats, a symposium sponsored by the Quartermaster Food and Container Institute for the Armed Forces.
21. HALVORSON, H. O. 1955. Factors determining survival and development of *Clostridium botulinum* in semi-preserved meats. Ann. Inst. Pasteur Lille **7**:53–67.
22. HANSEN, P.-I. E. 1964. Irradiation of meat products. Food Irrad. **4**(3):A10-A17.
23. HANSEN, N.-H., AND K. WARNOE. 1960. The keeping quality of canned ham treated with combinations of heat processing and irradiation. Riso Rept. No. 16, p. 53–55.
24. HESSEN, I., AND H. RIEMANN. 1959. Sporeforming bacteria in canned meats. Roy. Vet. Agri. Coll. Copenhagen, Den. Yearbook, p. 50–69.
25. INGRAM, M., AND B. C. HOBBS. 1954. The bacteriology of "pasteurized" canned hams. J. Roy. Sanit. Inst. **74**:1151–1163.
26. JENSEN, L. B., 1954. Microbiology of meats, 3rd. ed. Garrard Press, Champaign, Ill.
27. KEMPE, L. L., AND J. T. GRAIKOSKI. 1964. Effect of gamma irradiation on the spoilage of canned pork luncheon meat. Food Technol. **18**:134–136.
28. KNUDSEN, E. S. 1960. Radiation preservation of ham, luncheon meat, and bacon. Riso Rept. No. 16, p. 51–53.
29. KRABBENHOFT, K. L., D. A. CORLETT, JR., A. W. ANDERSON, AND P. R. ELLIKER. 1964. Chemical sensitization of *Clostridium botulinum* spores to radiation in meat. Appl. Microbiol. **12**:424–427.
30. MUNDT, J. O., AND H. M. KITCHEN. 1951. Taint in southern country-style hams. Food Res. **16**: 233–238.
31. MUNDT, J. O., C. J. MAYHEW, AND G. STEWART. 1954. Germination of spores in meats during cure. Food Technol. **8**:435–436.
32. OSHEROFF, B. J., G. G. SLOCUM, AND W. M. DECKER. 1964. Status of botulism in the United States. Publ. Health Rept. U.S. **79**:871–878.
33. PEDERSEN, H. O. 1958. The survival of *Clostridium botulinum* in curing brines. Proc. Intern. Symp. Food Microbiol., 2nd, Cambridge, Engl., 1957, p. 289–293.
34. PEDERSON, C. S. 1952. The inhibition of the activity of microorganisms in food products for the Armed Forces. Activities Rept., CMF&CI **4**:98–105.
35. PIVNICK, H., AND H. BARNETT. 1965. Effect of salt and temperature on toxinogenesis by *Clostridium botulinum* in perishable cooked meats vacuum-packed in air-impermeable plastic pouches. Food Technol. **19**:140–143.
36. PIVNICK, H., AND H. BIRD. 1965. Toxinogenesis by *Clostridium botulinum* Types A and E in perishable cooked meats vacuum-packed in plastic pouches. Food Technol. **19**:132–140.
37. RIEMANN, H. 1960. The effect of heating and irradiation on spores of a putrefactive anaerobe in phosphate buffer and in canned meat. Nord. Veterinarmed. **12**:86–104.
38. RIEMANN, H. 1963. Safe heat processing of canned cured meats with regard to bacterial spores. Food Technol. **17**:39–42, 45–56, 49.
39. ROBERTS, T. A., P. J. DITCHETT, AND M. INGRAM. 1965. The effect of sodium chloride on radiation resistance and recovery of irradiated anaerobic spores. J. Appl. Bacteriol. **28**:336–348.
40. ROBERTS, T. A., AND M. INGRAM. 1965. Radiation resistance of spores of *Clostridium* species in aqueous suspension. J. Food Sci. **30**:879–885.
41. SCHACK, W. R., R. A. GREENBERG, G. A. BLODGET, AND J. H. SILLIKER. 1958. Thermal processing characteristics of canned non comminuted meats. Food Res. **24**:112–118.
42. SCHMIDT, C. F. 1963. Appendix II. Dose requirements for the radiation sterilization of food. J. Appl. Radiation Isotopes **14**:19–26.
43. Schmidt, C. F., AND W. K. NANK. 1960. Radiation sterilization of food. I. Procedures for the evaluation of the radiation resistance of spores of *Clostridium botulinum* in food products. Food Res. **25**:321–327.
44. SCHMIDT, C. F., AND W. P. SEGNER. 1964. The bacteriology of type E *Clostridium botulinum*. Proc. Res. Conf., Am. Meat Inst. Found., 16th p. 13–20.
45. SCOTT, W. J. 1955. Factors in canned ham controlling *Clostridium botulinum* and *Staphylococcus aureus*. Ann. Inst. Pasteur Lille **7**:68–73.
46. SEGNER, W. P., C. F. SCHMIDT, AND J. K. BOLTZ. 1966. The effect of sodium chloride and pH on the outgrowth of spores of type E *Clostridium*

botulinum at optimal and suboptimal temperatures. Appl. Microbiol. **14**:49–54.

47. SILLIKER, J. H. 1959. The effect of curing salts on bacterial spores. Proc. Res. Conf. Am. Meat Inst. Found., 11th, p. 51–60.
48. SILLIKER, J. H., R. A. GREENBERG, AND W. R. SCHACK. 1958. Effects of individual curing ingredients on the shelf stability of canned comminuted meats. Food Technol. **12**:551–554.
49. STEINKE, P. K. W., AND E. M. FOSTER. 1951. Botulinum toxin formation in liver sausage. Food Res. **16**:477–484.
50. STEINKRAUS, K. H., AND J. C. AYRES. 1964. Incidence of putrefactive anaerobic spores in meat. J. Food Sci. **29**:87–93.
51. WYNNE, E. S., N. R. SCHMIEDING, AND G. T. DAYE, JR. 1955. A simplified medium for counting *Clostridium* spores. Food Res. **20**:9–12.