THE UNIVERSITY OF MICHIGAN RESEARCH INSTITUTE
ANN ARBOR, MICHIGAN

Progress Report

DETERMINATION OF RADIATION STERILIZATION DOSE FOR CANNED MEAT

L. L. Kempe
J. T. Graikoski

UMRI Project 2681

DEPARTMENT OF THE ARMY
QUARTERMASTER RESEARCH AND DEVELOPMENT COMMAND
QUARTERMASTER FOOD AND CONTAINER INSTITUTE
CONTRACT NO. DA-19-129-qm-964
CHICAGO, ILLINOIS

October 1958

CONTRACT RESEARCH PROJECT REPORT

QUARTERMASTER FOOD AND CONTAINER INSTITUTE
FOR THE ARMED FORCES, CHICAGO

Hq, QM Research and Development Command
QM Research and Development Center, Natick, Mass.

The University of Michigan
Research Institute
Ann Arbor, Michigan

Official Investigator: Lloyd L. Kempe
Collaborator: J. T. Graikoski

Project No. 7-84-01-002
Contract No. DA-19-129-qm-964
File No. S-510
Report No. 7 (Progress)
Period 1 August 1958 to
30 September 1958
Initiation Date: 1 August 1958

Title of Contract: Determination of Radiation Sterilization Dose for Canned Meat

SUMMARY

The irradiation sterilization dose for precooked ground beef inoculated with 5,000,000 Clostridium botulinum 213 B spores per gram of meat was found to be 3.8 megarad. Raw ground beef was found to require a like sterilizing dose when similarly inoculated. Also, cooked ground beef inoculated with approximately 5,000,000 C. botulinum 62 A spores per gram of meat was sterilized by 3.85 megarad of gamma radiation. Similar results were obtained with raw ground beef.

Studies of canned ground beef approximately 5 years old have been initiated to determine whether toxin could develop in these cans due to possible germination of "killed" spores with development of a few cells. No evidence of such toxin production has been found to date. At the same time, the meat in these cans has been tested for the presence of dormant spores. No cultures have developed in liver broth when such broth has been inoculated with 25-gm meat samples. There is, however, some suggestion of the development of a few vegetative cells in some instances.

DETERMINATION OF RADIATION STERILIZATION DOSE FOR CANNED MEAT

Previously reported data[1] showing the amount of gamma radiation required to sterilize ground beef packed in tin cans are being extended to include C. botulinum spore concentrations of at least one million per gram. These data will eventually include two strains of C. botulinum as well as data for cooked and raw ground beef. This report presents essentially completed data for both cooked and raw ground beef packed in mushroom-style tins and inoculated with C. botulinum 213 B spores, as well as some data for C. botulinum 62 A spores.

MATERIALS AND METHODS

Spores.—The C. botulinum spores used in this study were originally obtained from the Hooper Foundation for Medical Research at the University of California. C. botulinum 213 B spore suspensions were prepared according to procedures described by Reed et al.[2] except that Difco bacto-casitone was substituted for casein digest in the medium specified by these workers. The C. botulinum 62 A spores were grown in the liver broth medium described by Reed et al.[2] Stock spore suspensions were prepared in sterile distilled water, frozen, and then stored at -40°C until needed. Identity of the spores was verified by toxin neutralization tests of the culture media, as well as heat-resistance studies of the spores and the usual staining and microscopic controls. Appropriate dilutions for inoculation into canned meat were prepared after counting the viable spores present in the stock suspensions by the method of Reed.[2] For this purpose 0.1% soluble starch was incorporated into the pork agar medium to aid germination of the spores.[3]

Cooked Ground Beef.—Samples for irradiation were prepared from lean beef that was kept refrigerated during grinding and until used. For a run, the ground beef was placed in shallow enameled pans and cooked for 30 min at 15-lb steam pressure. Mushroom-type (202 x 202) cans were then filled within 1/4 in. of the top with hot meat, covered loosely by can lids, and sterilized at 121°C for 60 min. Individual cans were removed from the autoclave as needed, their covers were aseptically lifted, and 1 ml of a properly diluted spore suspension was injected into the geometrical center of the meat. This method of inoculation did not result in uniform spore distribution throughout the meat, but rather concentrated spores in the center of the can. Finally, the cans were sealed in a Western type of closing machine. Since the meat was still at a temperature of about 95°C, the cans were immersed in running tap water for 30 min, which cooled the meat to an average temperature of 20°C and produced a vacuum in the cans. The cans were then either cooled in a refrigerator to about 1°C or were immediately placed in the radiation room where they cooled to 5°C, or below, during irradiation.

For irradiation the cans were placed in the center well of the large cobalt-60 gamma irradiation source at the Fission Products Laboratory of The University of Michigan. During these experiments, the radiation dosage rate averaged about 120,000 rad per hour at the center of the cans.

Following irradiation, the cans were incubated at 29°C. Some of those that swelled were aseptically opened and subcultured to verify the C. botulinum culture growth. This verification also included both toxin presence and toxin neutralization tests in mice and was carried out on the meat from selected swollen cans as well as on culture media from the subcultures.

Raw Ground Beef.—Lean ground beef was spread into shallow enameled pans and placed in an evacuation chamber. Here dissolved metabolic gases and oxygen were removed by evacuation to 25 in. of Hg. This evacuation procedure was repeated three times, after which the meat was packed into muchroom-type (202 x 202) cans, inoculated, and sealed in a commercial-type vacuum closing machine under a 29-in.-Hg vacuum. The meat was kept below 40°F throughout this process. Experimental cans were then either irradiated, temporarily stored under refregeration, or incubated at 85°F as indicated.

RESULTS AND DISCUSSION

Cooked Ground Beef.—Data showing variation of the sterilization dose of gamma radiation for cooked ground beef as a function of spore concentration is tabulated in Table I, summarized in Table II, and plotted in Fig. 1. As would be expected from previously reported results,[1] the sterilizing dosage varies directly with the logarithm of the number of C. botulinum 213 B spores per gram of meat and the line shows a D value* of 0.34 megarad for these spores. A sterilization dose of 3.8 megarad of gamma radiation from cobalt-60 is indicated for cooked ground beef containing approximately 5,000,000 C. botulinum 213 B spores per gram. Since C. botulinum 213 B spores were previously[7] found to be slightly less resistant to gamma radiation than C. botulinum 62 A spores, it is reasonable to expect the sterilization dose based on the latter spores to be a few tenths of a megarad higher.

Raw Ground Beef.—It will be noted that, in Fig. 2 and Table IV, the radiation sterilization dose for ground beef, canned in the raw condition, also varies with the logarithm of the number of spores present. At a spore concentration of approximately one million C. botulinum 213 B spores per gram of raw ground beef, the sterilization dose is indicated as 3.6 megarad of gamma radiation from cobalt-60. It will be observed that the data for raw ground beef are less precise than those shown in Fig. 1 for cooked beef. This, in our opinion, is caused by the native bacterial flora of raw ground meat.

*The D value is the time in minutes required to reduce the number of viable spores by 90%.

The radiation sterilization dosage for both cooked and raw ground beef, inoculated with C. botulinum 62 A spores, is shown in Table III and summarized in Table II. These data indicate that cooked ground beef, inoculated with approximately 5,000,000 C. botulinum 62 A spores per gram, was sterilized by 3.85 megarad of gamma radiation. Similar results were obtained with raw ground beef under these conditions.

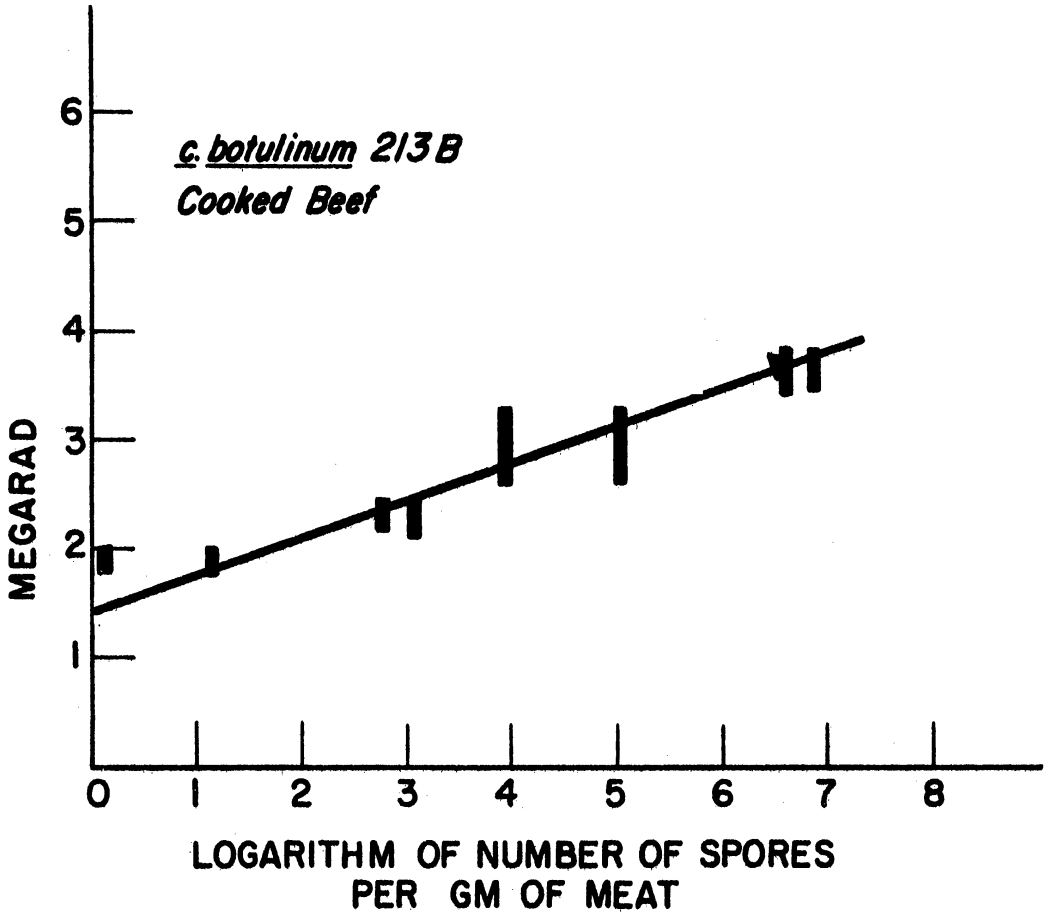

Fig. 1. The dosage of gamma radiation from cobalt-60 required to sterilize cooked ground beef containing spores of C. botulinum 213B.

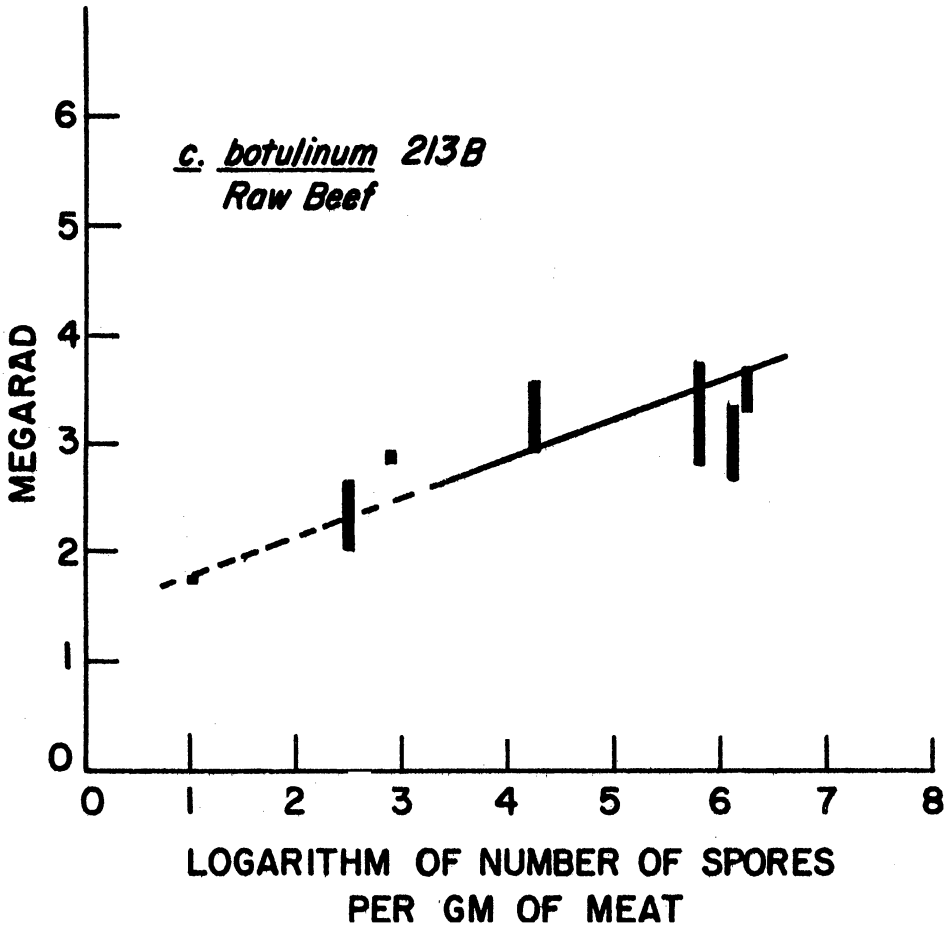

Fig. 2. The dosage of gamma radiation from cobalt-60 required to sterilize raw ground beef containing spores of C. botulinum 213B.

TABLE I. THE DOSAGES OF GAMMA RADIATION FROM COBALT-60 REQUIRED TO STERILIZE COOKED GROUND BEEF CONTAINING SPORES OF C. BOTULINUM 213B

Run No.: C-1
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 104,000 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
2.36	1	-
	2	-
	3	-
	4	-
	5	12
2.79	6	-
	7	13
	8	12
	9	-
	10	12
3.29	11	-
	12	-
	13	-
	14	-
	15	-
3.72	16	-
	17	-
	18	-
	19	-
	20	-
Noninoculated Controls	NI-1	-
	NI-2	-
	NI-3	-
	NI-4	-
Inoculated Controls	IC-1	5
	IC-2	5
	IC-3	5
	IC-4	5

Conclusion: Under these conditions cooked ground beef was sterilized with between 2.79 and 3.29 megarad of gamma radiation.

Run No.: C-2
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 8,600 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
2.36	1	12
	2	12
	3	12
	4	12
	5	12
2.79	6	14
	7	12
	8	11
	9	11
	10	12
3.29	11	-
	12	-
	13	-
	14	-
	15	-
3.72	16	-
	17	-
	18	-
	19	-
	20	-
Noninoculated Controls	NI-1	-
	NI-2	-
	NI-3	-
	NI-4	-
	NI-5	-
Inoculated Controls	I-1	4
	I-2	5
	I-3	5
	I-4	5
	I-5	5

Conclusion: Under these conditions cooked ground beef was sterilized with between 2.79 and 3.29 megarad of gamma radiation.

Run No.: C-3
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 1220 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
1.86	1	12	0/2
	2	-	
	3	13	2/2
	4	13	
	5	12	
2.10	6	-	
	7	13	
	8	11	
	9	11	2/2
	10	11	
2.46	11	-	
	12	-	
	13	-	
	14	-	
	15	-	
2.79	16	-	
	17	-	
	18	-	
	19	-	
	20	-	
Noninoculated Controls	NI-1	10	0/2
	NI-2	2	
	NI-3	10	0/2
	NI-4	11	
	NI-5	11	
Inoculated Controls	I-1	4	
	I-2	5	
	I-3	5	2/2
	I-4	5	
	I-5	-	

Conclusion: Under these conditions cooked ground beef was sterilized with between 2.10 and 2.46 megarad of gamma radiation.

Run No.: C-4
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 570 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
1.675	1	14	
	2	14	
	3	14	
	4	14	
	5	14	
1.86	6	12	
	7	-	
	8	11	
	9	12	
	10	11	
2.14	11	-	
	12	-	
	13	15	2/2
	14	-	
	15	-	
2.42	16	-	
	17	-	
	18	-	
	19	-	
	20	-	
Noninoculated Controls	NI-1	-	
	NI-2	-	
	NI-3	-	
	NI-4	-	
Inoculated Controls	IC-1	5	
	IC-2	5	
	IC-3	5	
	IC-4	5	

Conclusion: Under these conditions cooked ground beef was sterilized with between 2.14 and 2.42 megarad of gamma radiation.

Run No.: C-5
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 4,900,000 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
2.74	6	-	
	7	13	2/2
	8	18	2/2
	9	13	2/2
	10	-	
5.21	1	-	
	2	-	
	3	-	
	4	-	
	5	-	
5.86	11	-	
	12	-	
	13	-	
	14	-	
	15	-	
7.90	16	-	
	17	-	
	18	-	
	19	-	
	20	-	
Noninoculated Controls	NI-1	-	
	NI-2	-	
	NI-3	8	
	NI-4	-	
	NI-5	5	
Inoculated Controls	IC-1	5	
	IC-2	5	
	IC-3	5	
	IC-4	5	
	IC-5	5	

Conclusion: Under these conditions cooked ground beef was sterilized with between 2.74 and 5.21 megarad of gamma radiation.

Run No.: C-6
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 3,800,000 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
3.71	11	-	
	12	-	
	13	-	
	14	-	
	15	-	
4.19	16	-	
	17	-	
	18	-	
	19	-	
	20	-	
5.06	6	-	
	7	-	
	8	-	
	9	-	
	10	-	
5.62	1	-	
	2	-	
	3	-	
	4	-	
	5	-	
5.81	21	-	
	22	-	
	23	-	
	24	-	
	25	-	
Noninoculated Controls	B-1	-	
	B-2	-	
	B-3	13	0/2
	B-4	-	
	B-5	-	
Inoculated Controls	A-1	4	
	A-2	4	
	A-3	4	
	A-4	4	
	A-5	-	

Conclusion: Under these conditions cooked ground beef was sterilized with 3.71 megarad or less of gamma radiation.

Run No.: C-7
Can Size: Mushroom (202 x 202)
Production: Cooked ground beef
Inoculum: 4 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
1.395	17	7	
	18	7	
	19	7	
	20	9	
	21	9	
1.86	11	12	2/2
	12	12	
	13	-	
	14	12	
	15	13	
	16	10	
2.355	6	-	
	7	14	2/2
	8	-	
	9	-	
	10	-	
2.66	1	-	
	2	11	
	3	12	
	4	-	
	5	14	
Noninoculated Controls	NI-1	-	
	NI-2	-	
	NI-3	-	
	NI-4	-	
	NI-5	-	
Inoculated Controls	IC-1	5	
	IC-2	6	

Conclusion: Under these conditions cooked ground beef was not sterilized with up to 2.66 megarad of gamma radiation.

Run No.: C-8
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 4,000,000 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
3.88	1	-
	2	-
	3	-
	4	-
	5	-
	6	-
Inoculated Control	INC-1	3
	INC-2	3
	INC-3	4

Conclusion: Under these conditions cooked ground beef was sterilized with 3.88 megarad of gamma radiation or less.

Run No.: C-9
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 6,600,000 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
3.14	11	10	2/2
	12	-	
	13	10	2/2
	14	-	
	15	-	
3.42	6	-	
	7	-	
	8	15	2/2
	9	-	
	10	-	
3.86	1	-	
	2	-	
	3	-	
	4	-	
	5	-	
Noninoculated Controls	NI-1	-	
	NI-2	-	
	NI-3	-	
Inoculated Controls	INC-1	3	
	INC-2	3	

Conclusion: Under these conditions cooked ground beef was sterilized with between 3.42 and 3.86 megarad of gamma radiation.

Run No.: C-10
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 16.7 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
1.77	15	-	
	16	-	
	17	22	3/3
	18	-	
	19	-	
	20	-	
2.00	11	-	
	12	-	
	13	-	
	14	-	
2.39	6	-	
	7	-	
	8	-	
	9	-	
	10	-	
2.70	1	-	
	2	-	
	3	-	
	4	-	
	5	-	
Noninoculated Controls	NIC-1	-	
	NIC-2	-	
	NIC-3	-	
	NIC-4	-	
Inoculated Controls	INC-1	9	2/2
	INC-2	9	2/2

Conclusion: Under these conditions cooked ground beef was sterilized with between 1.77 and 2.00 megarad of gamma radiation.

Run No.: C-11
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 1.42 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
1.00	6	-
	7	11
	8	12
	9	12
	10	12
1.40	1	14
	2	-
	3	12
	4	-
	5	11
1.80	21	-
	22	-
	23	13
	24	-
	25	-
2.00	11	-
	12	-
	13	-
	14	-
	15	-
2.50	16	-
	17	-
	18	-
	19	-
	20	-
Noninoculated Controls	NIC-1	-
	NIC-2	-
Inoculated Controls	INC-1	11
	INC-2	11
	INC-3	11

Conclusion: Under these conditions cooked ground beef was sterilized with between 1.80 and 2.00 megarad of gamma radiation.

TABLE II. SUMMARY OF DOSAGES OF GAMMA RADIATION FROM COBALT-60 REQUIRED TO STERILIZE GROUND BEEF CONTAINING SPORES OF C. BOTULINUM

Run No.	No. of Spores per gm of Meat	Radiation Sterilization Range, Megarad
A. C. botulinum 62A spores in cooked ground beef		
AC-2	4,800,000	3.50-3.80
AC-1	5,200,000	3.40-3.85
B. C. botulinum 62A spores in raw ground beef		
A-4	1,330,000	
A-2	2,670,000	3.20-3.60
A-3	3,200,000	Slightly more than 3.80
C. C. botulinum 213B spores in cooked ground beef		
C-11	1.42	1.80-2.00
C-7	4.00	>2.66
C-10	16.7	1.77-2.00
C-4	570	2.14-2.42
C-3	1,220	2.10-2.46
C-2	8,600	2.79-3.29
C-1	104,000	2.79-3.29
C-6	3,880,000	<3.71
C-8	4,000,000	<3.88
C-5	4,900,000	2.74-5.21
C-9	6,600,000	3.42-3.86
D. C. botulinum 213B spores in raw ground beef		
S-4	10.9	1.70-1.75
S-7	311	2.00-2.65
S-5	790	2.80-2.90
S-3	17,000	2.90-3.53
S-1	632,000	2.79-3.72
S-6	1,440,000	2.65-3.30
S-2	1,700,000	3.29-3.72

TABLE III. THE DOSAGES OF GAMMA RADIATION FROM COBALT-60 REQUIRED TO STERILIZE GROUND BEEF INOCULATED WITH APPROXIMATELY 1,000,000 C. BOTULINUM 62A SPORES PER GRAM

Run No.	AC-1
Can Size:	Mushroom (202 x 202)
Product:	Cooked ground beef
Inoculum	5,200,000 C. botulinum 62A spores per gm of meat
Incubation Temperature:	85°F

A. C. botulinum 62A spores in cooked ground beef

Megarad	Can No.	Days-to-Gas Formation
2.90	21	3
	22	3
	23	3
	24	4
	25	4
	31	3
	32	4
	33	5
3.40	16	9
	17	-
	18	-
	19	5
	20	-
3.85	26	-
	27	-
	28	-
	29	-
	30	-
4.10	1	-
	2	-
	3	-
	4	-
	5	-
4.80	6	-
	7	-
	8	-
	9	-
	10	-
Noninoculated Controls	NI-1	-
	NI-2	-
	NI-3	-
	NI-4	-
	NI-5	-
Inoculated Controls	IC-1	2
	IC-2	2
	IC-3	2
	IC-4	2
	IC-5	2

Conclusion: Under these conditions cooked ground beef was sterilized by between 3.40 and 3.85 megarad of gamma radiation.

Run No.: AC-2
Can Size: Mushroom (202 x 202)
Product: Cooked ground beef
Inoculum: 4,800,000 C. botulinum 62A spores per gm of meat
Incubation Temperature: 85°F

A. C. botulinum 62A spores in cooked ground beef

Megarad	Can No.	Days-to-Gas Formation
2.75	16	4
	17	16
	18	5
	19	4
	20	-
3.00	21	5
	22	5
	23	-
	24	4
	25	5
3.25	26	6
	27	5
	28	10
	29	5
	30	-
3.50	11	-
	12	-
	13	5
	14	5
	15	7
3.80	1	-
	2	-
	3	-
	4	-
	5	-
4.15	6	-
	7	-
	8	-
	9	-
	10	-
Noninoculated Controls	NI-1	-
	NI-2	7
	NI-3	-
	NI-4	-
	NI-5	15
Inoculated Controls	INC-1	3
	INC-2	4

Conclusion: Under these conditions cooked ground beef was sterilized by between 3.50 and 3.80 megarad of gamma radiation.

Run No.: A-1
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 670,000 C. botulinum 62A spores per gm of meat
Incubation Temperature: 85°F

B. C. botulinum 62A spores in raw ground beef

Megarad	Can No.	Days-to-Gas Formation
3.20	A-21	-
	A-22	-
	A-23	-
	A-24	-
	A-25	-
3.35	A-16	-
	A-17	-
	A-18	-
	A-19	-
	A-20	-
3.65	A-26	-
	A-27	-
	A-28	-
	A-29	-
	A-30	-
4.35	A-1	-
	A-2	-
	A-3	-
	A-4	-
	A-5	-
4.60	A-6	-
	A-7	-
	A-8	-
	A-9	-
	A-10	-
5.30	A-11	-
	A-12	-
	A-13	-
	A-14	-
	A-15	-

Remarks: These were old spores grown and harvested from trypticase broth two years ago and kept at 40°F in distilled water in the interim.
Conclusion: None.

Run No.: A-2
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 2,670,000 C. botulinum 62A spores per gm of meat
Incubation Temperature: 85°F

B. C. botulinum 62A spores in raw ground beef

Megarad	Can No.	Days-to-Gas Formation
3.20	11	5
	12	5
	13	4
	14	-
	15	-
3.60	1	-
	2	-
	3	-
	4	-
	5	-
4.90	6	-
	7	-
	8	-
	9	-
	10	-
Noninoculated Controls	1	4
Inoculated Controls	1	3
	2	3
	3	3
	4	3

Conclusion: Under these conditions raw ground beef was sterilized by between 3.20 and 3.60 megarad of gamma radiation.

Run No.: A-3
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 3,200,000 C. botulinum 62A spores per gm of meat
Incubation Temperature: 85°F

B. C. botulinum 62A spores in raw ground beef

Megarad	Can No.	Days-to-Gas Formation
2.70	16	5
	17	5
	18	5
	19	5
	20	6
3.15	21	4
	22	4
	23	4
	24	4
	25	4
3.50	11	-
	12	-
	13	6
	14	-
	15	6
	1	5
	2	5
	3	5
	4	5
	5	6
3.80	6	-
	7	-
	8	-
	9	-
	10	-
	26	-
	27	5
	28	6
	29	-
	30	-
Noninoculated Controls	NI-1	1
	NI-2	1
Inoculated Controls	IC-1	1

Conclusion: Under these conditions cooked ground beef was not sterilized by 3.80 megarad of gamma radiation although the sterility dose for this spore concentration appears to be only slightly greater than this level.

Run No. A-4
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 1,330,000 C. botulinum 62A spores per gm of meat
Incubation Temperature: 85°F

B. C. botulinum 62A spores in raw ground beef

Megarad	Can No.	Days-to-Gas Formation
3.30	16	-
	17	-
	18	-
	19	-
	20	-
3.60	11	-
	12	-
	13	-
	14	-
	15	-
3.80	1	-
	2	-
	3	-
	4	-
	5	-
4.20	6	-
	7	-
	8	-
	9	-
Inoculated Controls	IC-1	1
	IC-2	2

Conclusion. None.

C. C. botulinum 213B spores in cooked ground beef

See Table II, Runs C-5, C-6, C-8, and C-11.

D. C. botulinum 213B spores in raw ground beef

See Table IV, Runs S-1 and S-2.

TABLE IV. THE DOSAGES OF GAMMA RADIATION REQUIRED TO STERILIZE RAW GROUND BEEF INOCULATED WITH C. BOTULINUM 213B SPORES

Run No.: S-1
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 632,000 spores per gm of meat
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
2.79	1*	-	0/2
	2	5	0/2
	3*	-	0/2
	4	-	
	5	-	
3.72	6	-	
	7	-	
	8	-	
	9	-	
	10	-	
4.83	11	-	
	12	-	
	13*	-	0/2
	14	-	
	15	-	
5.58	16*	-	0/2
	17	-	
	18	-	
	19	-	
	20	-	
Noninoculated Controls	NI-1	2	
	NI-2	2	
Inoculated Controls	INC-1	2	0/2
	INC-2	1	0/2

*Tested for toxin even though the cans were not swollen sufficiently to be positive for gas.

Conclusion: Under these conditions raw ground beef was sterilized by between 2.79 and 3.72 megarad of gamma radiation.

Run No.: S-2
Can Size: Mushroom (202 x 202)
Production: Raw ground beef
Inoculum 1,700,000 spores per gm of meat
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin Production
2.18	S-17	3	
	S-18	3	
	S-19	3	
2.48	S-20	3	3/4
	S-21	3	
	S-22	3	
	S-23	3	
	S-24	3	
2.79	S-1	3	
	S-2	3	
	S-3	3	
	S-4	2	
	S-5	3	
	S-6	3	
3.29	S-12	3	0/2
	S-13	4	
	S-14	3	
	S-15	3	0/2
	S-16	3	
3.72	S-7	-	
	S-8	-	
	S-9	-	
	S-10	-	
	S-11	-	
Noninoculated Controls	NI-1	2	
	NI-2	5	
	NI-3	5	
	NI-4	4	

Remarks: A portion of meat from can S-7 was aseptically removed and inoculated into pea-pork infusion media. No growth resulted. This is additional evidence of sterility at 3.72 megarad.

Conclusion: Under these conditions, raw ground beef was sterilized by between 3.29 and 3.72 megarad of gamma radiation.

Run No.1 S-3
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 17,000 spores per gm of meat
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
2.56	16	3
	17	3
	18	-
	19	4
	20	-
2.90	11	3
	12	3
	13	3
	14	3
	15	3
3.53	6	-
	7	-
	8	-
	9	-
	10	-
4.00	1	-
	2	-
	3	-
	4	-
	5	-
Noninoculated Controls	NI-1	1
	NI-2	1
	NI-3	1
	NI-4	1
	NI-5	1

Conclusion: Under these conditions raw ground beef was sterilized by between 2.90 and 3.53 megarad of gamma radiation.

Run No.: S-4
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 10.9 C. botulinum 213B spores per gm of meat
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
1.00	21	4
	22	5
	23	5
	24	4
	25	4
1.40	26	5
	27	4
	28	5
	29	5
	30	4
1.50	31	4
	32	4
	33	5
	34	5
	35	5
1.70	36	4
	37	-
	38	5
	39	5
	40	4
1.75	16	-
	17	-
	18	-
	19	-
	20	-
2.40	1	-
	2	-
	3	-
	4	-
	5	-
	11	-
	12	-
	13	-
	14	-
	15	-
2.70	6	-
	7	-
	8	-
	9	-
	10	-

Conclusion: Under these conditions raw ground beef was sterilized by between 1.70 and 1.75 megarad of gamma radiation.

Run No.: S-5
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 790 C. botulinum 213B spores per gm
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
1.90	16	4
	17	6
	18	4
	19	4
	20	-
2.10	21	-
	22	-
	23	5
	24	-
	25	-
2.70	1	-
	2	-
	3	-
	4	-
	5	-
	11	-
	12	-
	13	-
	14	-
	15	-
2.80	26	5
	27	-
	28	-
	29	-
	30	-
2.90	6	-
	7	-
	8	-
	9	-
	10	-
3.40	36	-
	37	-
	38	-
	39	-
	40	-
3.75	31	-
	32	-
	33	-
	34	-
	35	-
Noninoculated Controls	NIC-1	2
	NIC-2	3
	NIC-3	3

Conclusion: Under these conditions raw ground beef was sterilized by between 2.8 and 2.9 megarad of gamma radiation.

The "skip" observed here has been found before with raw ground beef which, of course, has a natural bacterial flora of unknown quality and quantity.

Run No.: S-6
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 1,440,000 C. botulinum 213B spores per gm of meat
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
2.65	21	-
	22	5
	23	5
	24	4
	25	5
3.30	16	-
	17	-
	18	-
	19	-
	20	-
3.65	26	-
	27	-
	28	-
	29	-
	30	-
	36	-
	37	-
	38	-
	39	-
	40	-
4.00	31	-
	32	-
	33	-
	34	-
	35	-
4.65	1	-
	2	-
	3	-
	4	-
	5	-
	11	-
	12	-
	13	-
	14	-
	15	-
5.00	6	-
	7	-
	8	-
	9	-
	10	-

Conclusion: Under these conditions raw ground beef was sterilized by between 2.65 and 3.30 megarad of gamma radiation.

Run No.: S-7
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 311 C. botulinum 213B spores per gm of meat
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
1.00	21	4
	22	3
	23	4
	24	5
	25	3
1.40	26	5
	27	5
	28	4
	29	3
	30	4
2.00	31	-
	32	6
	33	6
	34	4
	35	5
2.65	16	-
	17	-
	18	-
	19	-
	20	-
3.20	36	-
	37	-
	38	-
	39	-
	40	-
3.65	1	-
	2	-
	3	-
	4	-
	5	-
	11	-
	12	-
	13	-
	14	-
	15	-
4.00	6	-
	7	-
	8	-
	9	-

Conclusion: Under these conditions raw ground beef was sterilized by between 2.00 and 2.65 megarad of gamma radiation.

STUDIES OF STORED CANS OF MEAT STERILIZED IN PREVIOUS YEARS BY GAMMA RADIATION

Inoculated cans of ground beef that had previously been sterilized by gamma radiation have been stored in our laboratory for several years. Some of these cans were prepared as long ago as 1953. Since the time that these cans were processed, many changes could have occurred in the meat contained in them. It seems pertinent to learn whether spores that were "killed" by ionizing radiation could have germinated and evolved through a few divisions and thus developed enough toxin to be discoverable. This seems possible, but not likely. However, it should be evaluated. Also, there is the possibility that spores of C. botulinum could remain alive but ungerminated in the meat and that these spores could be revived by subculture. Some of these cans of radiation-sterilized meat are being aseptically opened and the meat is being tested for the presence of botulinus toxin and dormant spores.

MATERIALS AND METHODS

Canned meat for analysis is selected from those cans which have been stored the longest and of these, those cans remaining from groups that received the minimum radiation sterilizing dose are preferred.

The cans are first washed with detergent and water, then they are dried, placed in an enameled pan on a towel soaked in a cresylone solution, and a depression in the top of the can is filled with 95% ethyl alcohol. This is then ignited and allowed to burn off. A sterile pad of cotton is then aseptically placed over the can in preparation for releasing the vacuum. This is accomplished by punching a small hole in the can cover with an ice pick. This instrument is first sterilized in a gas flame. It is then pushed through the cotton pad and into the can. Air that now enters the can should be drawn through the cotton filter. The can cover is now removed with a hand can opener that was previously sterilized in a steam heater autoclave.

Four samples of meat are now taken. These are removed with a sterile tube and plunger-type sampler. Three of these are used for subcultures and one for toxin analysis. The three for subculturing weigh approximately 15 g each and the sample for toxin analysis is about twice as large.

For subculturing, each of the 15-g samples is pushed into individual tubes of N.C.A. liver broth containing a strip of pure iron. These tubes of broth are first exhausted in a hot water bath. They contain 50 ml of media. The tubes of broth and meat are now incubated for 2 weeks at 85°F unless visible growth occurs before this time has elapsed. Following either the evident development of a culture or the 2-week interval, the liquid in the tube is examined for the presence of bacteria. For this purpose, Gram stains are prepared

and examined. If any growth is evident, the liquid is further tested for the presence of toxin by injecting a portion into four 10- to 15-gm mice. Should it become necessary, further studies are carried out involving intraperitoneal injection of portions of the sample incubated overnight in the ice box with specific-type botulinus antitoxin into mice.

The larger, approximately 25-g sample is pushed into a sterile test tube and then an approximately equal volume of physiological saline is added. The meat and saline are aseptically mixed and then allowed to infuse in a refrigerator for a few hours. The supernatant liquid is then aseptically filtered through a glass-wool pad. Then 1/2-ml portions of the filtrate are injected intraperitoneally into each of four 10- to 15-gm mice. If no mice die within two weeks, the sample is assumed to be nontoxic. Should one or more mice die, a portion of the filtrate is mixed with the specific-type botulinus antitoxin and this mixture is again injected into mice for final determination of toxigenicity of the filtrate.

RESULTS

Description of Samples for Analysis.—For Group 1, six No. 2 cans of ground beef were removed from room-temperature storage. These were irradiation-sterilized late in 1953 and have been in our laboratory since that time. When opened, all the cans had a considerable vacuum. The meat did not have any unusual odor and looked like cooked hamburger, which it was. It should be pointed out that these cans of meat were sterilized in a steam-heated autoclave and then inoculated before irradiation sterilization. The process used for this purpose has been published.[1]

Group 2 is similar to Group 1.

The data presented in Tables V and VI so far indicate that none of the irradiation-sterilized cans of ground beef that have been stored in our laboratory contains botulinus or other toxin that would kill mice on intraperitoneal injection. The finding of a few Gram-positive rads in the subculture suggests that some of the irradiation-"killed" spores may germinate and develop a few vegetative cells. This is only suggested by these data, however. In any event, the limited data so far available suggest that, if such cells do develop, they do not liberate enough toxin into the broth to make it toxic for mice according to our test procedure.

TABLE V. DESCRIPTION OF SAMPLES USED FOR DETERMINATION OF THE POSSIBLE PRESENCE OF BOTULINUS TOXIN ON DORMANT C. BOTULINUM SPORES IN IRRADIATION-STERILIZED CANNED GROUND BEEF

Code Designation of the Can	Date Irradiated	Irradiation Dose, megarep	Type of C. botulinum Spores	No. of Spores per Gram
		Group 1		
H2.5B	11/12/53	2.549	62A	4,000
H3.0B	11/12/53	2.683	62A	4,000
H3.5B(1)	11/12/53	3.576	62A	4,000
H3.5B(2)	11/12/53	3.576	62A	4,000
BB3.5(1)	12/11/53	3.494	213B	40,000
BB3.5(2)	12/11/53	3.494	213B	40,000
		Group 2		
X-2-B(1)	11/13/53	2.027	62A	4
X-2-B(2)	11/13/53	2.027	62A	4
X-2.5B	11/13/53	2.424	62A	4
X-NI	11/13/53	None	Not Inoculated	

TABLE VI. DETERMINATION OF THE POSSIBLE PRESENCE OF BOTULINUS TOXIN OR DORMANT C. BOTULINUM SPORES IN IRRADIATION-STERILIZED CANNED GROUND BEEF

Code Designation of Can	Toxicity Test of Meat*	Growth in Liver Broth	Gram Stain of Liver Broth	Toxicity Test of Liver Broth*
		Group 1		
H2.5B	0/4	1 Not apparent 2 Not apparent 3 Not apparent	1 Few Gm (+) rods 2 Few Gm (+) rods 3 Few Gm (+) rods	0/2
H3.0B	0/4	1 Not apparent 2 Not apparent 3 Not apparent	1 Few Gm (+) rods 2 Few Gm (+) rods 3 Few Gm (+) rods	0/2
H3.5B(1)	0/4	1 Not apparent 2 Not apparent 3 Not apparent	1 Few Gm (+) rods 2 Few Gm (+) rods 3 Few Gm (+) rods	0/2
H3.5B(2)	0/4	1 Not apparent 2 Not apparent 3 Not apparent	1 0 2 0 3 0	0/2
H3.5(1)	0/4	1 Not apparent 2 Not apparent 3 Not apparent	1 0 2 0 3 0	0/2
H3.5(2)	0/4	1 Not apparent 2 Not apparent 3 Not apparent	1 0 2 0 3 0	0/2
		Group 2		
X-2-B(1)	0/3	1 Not apparent 2 Not apparent 3 Not apparent	1 0 2 0 3 0	
X-2-B(2)	0/3	1 Not apparent 2 Not apparent 3 Not apparent	1 0 2 0 3 0	
X-2.5B(1)	0/3	1 Not apparent 2 Not apparent 3 Not apparent	1 0 2 0 3 0	

*This ratio refers to number of mice dying as compared to the number inoculated, i.e., 0/4 means that none of the four inoculated mice died.

REFERENCES

1. Kempe, L. L., Graikoski, J. T., and Gillies, R. A., "Gamma ray sterilization of canned meat previously inoculated with anaerobic bacterial spores," Appl. Microbiol., 2, 330-332 (1954).

2. Reed, J. M., Bohrer, C. W., and Cameron, E. J., "Spore destruction rate studies on organisms of significance in the processing of canned foods," Food Research, 16, 383-408 (1951).

3. Wynne, E. S., and Foster, J. W., "Physiological studies on spore germination with special reference to Clostridium botulinum," J. Bacteriology, 55, 61-68 (1948).