ENGINEERING RESEARCH INSTITUTE
THE UNIVERSITY OF MICHIGAN
ANN ARBOR

Final Report

COMBINED USE OF HEAT AND RADIATION TREATMENT FOR STERILIZATION OF FOODS

June 7, 1955, to July 31, 1957

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CONTRACT RESEARCH PROJECT REPORT

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GENERAL SUMMARY

In order that irradiation processing of foods can be intelligently considered, basic factual information is needed. Such information includes the effects of temperature on the lethality of ionizing radiations for anaerobic bacterial spores that are important in food sterilization, the effects of medium components, as well as the combined effects of temperature and such radiations. Where it may be planned to use irradiation alone for processing foods, it is necessary to know whether or not such a treatment is effective at refrigerating or freezing temperatures which protect the food before irradiation. Further, do chemical additives used in the preparation of the food affect the sterilizing effectiveness of ionizing radiation? Does preirradiation change the Z value of anaerobic bacterial spores still remaining in the food? How is the combined irradiation-heat process affected by the type of food being processed?

These and other questions have provided the necessity for and objectives of this work.

The work has so far established the following:

1. An induction dosage of approximately one megarep of gamma radiation is required before the combined irradiation—heat processing of food shows advantage. For example, using 300 C. botulinum 213B spores in a No. 1 picnic can of ground beef, sterility was attained with an F_0 of 0.15 following 1.0 megarep of radiation. But, following 0.5 megarep or less, an F_0 of 0.4 was needed to produce sterile cans of meat.

2. A combined process sufficient to sterilize canned ground beef, inoculated with 300 PA 3679 spores per can is much more than adequate to sterilize similar meat containing 5,000,000 C. botulinum 213B spores per can. This provides a large margin of safety against possible botulinus poisoning from underprocessed meat, provided the process used is designed to kill PA 3679 spores.

3. There is little if any difference between the combined irradiation—heat processing treatments required to sterilize canned raw or canned cooked beef. Thus it would be equally acceptable to irradiate raw or cooked beef before applying the reduced heat processing treatment subsequently required for sterilization.

4. Based on one series of experiments the Z values of irradiated and nonirradiated PA 3679 spores appear to be the same when these spores are heat-processed in distilled water. Therefore, the F_0 calculations in this report appear to be justified.

5. C. botulinum 213B and PA 3679 spores suspended in phosphate buffer (pH 7.02) containing catalase were much less sensitive to the lethal action of gamma radiation than were similarly treated spores in the absence of catalase. This finding supports the theory that the lethal action of ionizing radiations is at least partially due to secondary effects of the irradiation. Furthermore, such protection of anaerobic bacterial spores is important when sterilization of foods containing catalase is considered since it will likely increase the dosage required.

6. Combined irradiation-heat processing studies of canned frozen peas are incomplete at present. Results will not be available for two or three months due to slow germination of PA 3679 spores in the pea brine. Results will be reported at a later time.

7. Reduced sulfhydryl groups present in anaerobic bacterial spores protect these spores against the lethal activities of gamma radiation, but oxidized sulfhydryl groups are ineffective.

8. Different anaerobic bacterial spores have different temperature ranges in which they are less sensitive to the lethal effects of gamma radiation. However, when such radiations are to be used alone for food sterilization, refrigeration or freezing temperatures are best suited for the process since both C. botulinum and PA 3679 spores are more sensitive to gamma radiation at low temperatures than they are at temperatures between 5°C and 95°C.

This is compatible with processing schedules designed to protect foods by refrigeration or freezing before and during irradiation.

9. Combined irradiation—heat processing of foods will involve heating the foods to at least 95°C, either subsequent to or during irradiation. This follows from the discovery that a critical temperature exists at about 95°C, below which irradiated anaerobic bacterial spores are no more sensitive to heat than are nonirradiated spores. However, above 95°C, previously irradiated spores are much more easily killed than are nonirradiated spores of C. botulinum or PA 3679.

Several papers are in preparation as a result of this work. They will include the following:

1. Combined irradiation—heat sterilization of canned ground beef: by Lloyd L. Kempe, J. T. Graikoski, and P. F. Bonventre. There will be at least three papers on this subject.

2. The effect of chemicals on the lethality of gamma radiation for anaerobic bacterial spores: by Nancy J. Williams and Lloyd L. Kempe.

3. The effect of temperature during irradiation on the lethality of gamma radiations for anaerobic bacterial spores: by J. T. Graikoski and Lloyd L. Kempe.

4. Sensitization of anaerobic bacterial spores to temperatures above 85°C by gamma radiation: by J. T. Graikoski and Lloyd L. Kempe.

OBJECTIVE

This work was undertaken to develop combined irradiation—heat processing schedules for canned food and to study the effect of environmental conditions on the lethality of gamma radiation from cobalt-60 for the spores of anaerobic bacteria that are significant in food spoilage.

PHASE I

EFFECT OF PREIRRADIATION OF INOCULATED PACKS OF CANNED GROUND BEEF ON THE F_0 SUBSEQUENTLY REQUIRED FOR STERILIZATION

SUMMARY

Data are presented to show that the F_0 required to sterilize inoculated packs of either raw or precooked ground beef can be considerably reduced by preirradiation with gamma rays from cobalt-60.

Ground beef packed in No. 1 picnic tin cans was sterilized by combined irradiation—heat treatments, as well as by heat and by irradiation processing alone. Results of these studies are summarized in the following table (on page 2).

It will be noted that a combined process, sufficient to sterilize precooked ground beef containing 300 PA 3679 spores per can, is much more than adequate to sterilize similar meat containing 5,000,000 C. botulinum 213B spores per can. This provides a large margin of safety against possible botulinus poisoning from underprocessed meat if the process is originally designed to kill PA 3679 spores.

The data adequately demonstrate little if any difference between cooked and naw meat with respect to the severity of irradiation—heat processing treatments required to produce sterile products.

INTRODUCTION

Combined irradiation—heat processing of canned, cooked, ground beef inoculated with C. botulinum 213B spores was previously shown to require less of these forms of energy when they were used together than when either form was used alone.[3] However, it was necessary to extend the studies to include bacterial spores of greater heat resistance than those of C. botulinum. Therefore the work was continued using PA 3679 spores. It was also considered desirable to determine whether the results obtained with cooked ground beef would be applicable to such meat preirradiated in this raw state. For this purpose, raw meat inoculated with C. botulinum and like packs inoculated with PA 3679 spores were tested in a manner similar to that reported for cooked ground beef.

This work, then, was designed to establish combined irradiation—heat processing treatments that would sterilize canned ground meat. From these treatments, it should be possible to select the most desirable combined

TREATMENTS REQUIRED TO STERILIZE CANNED GROUND BEEF PACKED IN NO. 1 PICNIC TIN CANS USING HEAT AND GAMMA RADIATION ALONE AND IN COMBINATION

Condition of Ground Beef During Irradiation	Kind of Spores	Number of Spores	Sterilization Conditions					
			Irradiation Only		Heat Only	A Combined Process		
						Preirradiation		Heat
			Approx. Megarep	Approx. Megarad	F_o	Megarep	Magarad	F_o
Precooked	C. botulinum 213B	5,000,000	3.4-3.9	3.2-3.6	1.0	1.2	1.12	0.20
Precooked	C. botulinum 213B	300	1.6-1.8	1.5-1.7	0.4	1.0	0.93	0.15
Precooked	PA 3679	10,000	2.1	2.0	7.0	1.9	1.77	1.0
Precooked	PA 3679	300	1.8-2.0	1.7-1.9	6.0	1.3	1.21	1.0
Raw	C. botulinum	5,000,000	2.8-3.0	2.6-2.8	1.1	1.2	1.12	0.2
Raw	PA 3679	300	2.0-2.3	1.9-2.1	6.0	1.3	1.21	1.0

process for future organoleptic and similar studies.

MATERIALS AND METHODS

a. Packing

1. Cooked Meat Packs.—Lean ground beef is purchased locally from The University of Michigan food stores. The meat is placed in shallow pans and autoclaved at 15 psig steam pressure for one-half hour. Excess liquid is poured off, and the hot meat is packed into No. 1 picnic tin cans, some of which have previously been fitted with O. F. Ecklund thermocouples. Covers are set loosely on the cans of meat which are then placed in an autocalve where they are sterilized at 17 psig steam pressure for one hour. Next, each can is removed individually from the hot autoclave and the meat is inoculated with 1 ml of a spore suspension. The cans are then sealed in a commercial-type closing machine, immersed in cold tap water for about 20 minutes, and placed in ice water for an hour.

2. Raw Meat Packs.—The cold, lean ground beef is spread out in shallow pans and then placed in an evacuation chamber. Here the dissolved metabolic gases are removed by evacuating to about 25 inches of mercury, after which the vacuum is released. This is repeated three times while the meat is in the pans. Next the ground beef is packed into No. 1 picnic tin cans, being careful to avoid air pockets. Some of the cans are equipped with Ecklund thermocouples. Now the cans are placed in the evacuation chamber and the gas exhaustion procedure previously described is again carried out three times. The meat remains very cold throughout this procedure. Following degassing, the meat is inoculated at approximately the geometrical center of the can with 1 ml of a spore suspension that has been prepared in distilled water. Finally the cans are sealed in a commercial-type vacuum closing machine at a vacuum of about 26 inches of mercury.

Experimental cans are then either irradiated or temporarily stored in a refrigerator, as required; controls are placed in an 85°F incubator immediately; processed cans are quickly cooled by immersion in cold water before incubation.

b. Irradiation

The canned meat is irradiated in the "center well" of the large cobalt-60 source here at The University of Michigan. The temperature of the meat is kept below 4°C during irradiation. Actual dosage delivered at the center of the can is measured by ferrous-feric sulfate dosimetry as previously described.[1]

c. Heat Processing

This process is carried out as follows:

1. A 3-gallon pail is positioned in the upper part of a steam-heated autoclave, and the pail is half filled with water at 180°F.

2. Six cans of meat, two of which contain thermocouples, are removed from the refrigerator, thermocouples leads are attached to the two control cans, and then all six cans are placed in the 180°F water. Temperature measurements of the water in the pail and in the center of each can containing thermocouples are begun immediately.

3. The autoclave cover is clamped shut, and steam is introduced at such a rate that the water surrounding the cans is maintained at 180°F until the two thermocouples in the cans of meat show identical temperatures of 170°F or more.

A temperature of 180°F was selected because (a) it represents "hot-filling" temperatures of industrial practice, (b) it is not sufficient to cause appreciable killing of spores anywhere in the can during temperature equilibration, and (c) it is high enough to make "come-up" rates to the processing temperature essentially uniform in all the cans.

4. When the cans have equilibrated at some temperature between 170°F and 180°F, the water-bath temperature is quickly brought to 230°F by introducing steam into the autoclave. Processing time to attain the desired F_0 value, less the cooling increment, is now provided. Note: A processing temperature of 250°F was first used (Runs PA-2 to PA-9) but this was found to be too high and 230°F was adopted instead.

5. At the proper time, the autoclave is quickly opened and the cans are plunged into ice water. Temperature measurements are continued until the temperature at the center of the cans reaches 180°F.

6. Experimental cans of meat are incubated at 85°F; the cans containing thermocouples are again refrigerated; they are used a second time only.

7. Four sets of cans are autoclaved for each run, using arbitrarily selected processing times designed to provide suitable F_0 increments. After the runs are completed, the actual F_0 accomplished for each set of four cans is computed. This, together with the incubation results, constitutes the basic data acquired.

8. F_0 values are calculated by O. T. Schultz's graphical modification of C. O. Ball's General Method. In these calculations the Z value of both irradiated and nonirradiated C. botulinum 213B spores is assumed to be 18.

d. Spores

The spores of anaerobic bacteria used in these studies are prepared and used according to techniques described in previously published work from this laboratory.[1]

RESULTS

Canned ground beef packed in No. 1 picnic tin cans was sterilized by combined irradiation—heat processing. Data from the previous year's work, showing suitable combined processes of this type for cooked ground beef inoculated with C. botulinum 213B spores, are included here for reference and for purposes of comparison. These data, shown in Figs. 1 and 2, indicate the following:

a. A preirradiation "induction" dosage was apparent, before sensitization of the spores to heat became significantly important.

b. The data in Fig. 1 show that when 5,000,000 C. botulinum 213B spores were used per can of meat, heat processing alone required an F_0 of approximately 1.0 for sterilization. Similarly, irradiation alone required between 3.4 and 3.9 megarep to produce sterility. But when preirradiation with 1.2 megarep of gamma radiation was used, sterility was attained with an F_0 of approximately 0.2.

c. From Fig. 2 it will be observed that when using 300 C. botulinum 213B spores per can of meat, sterilization was attained with heat alone using an F_0 of approximately 0.4, while with irradiation alone approximately 1.7 megarep of gamma radiation from cobalt-60 were required. On the other hand, when irradiation and heat processing were combined, sterility was attained with an F_0 of approximately 0.15 following 1.0 megarep of radiation.

This year the combined irradiation—heat processing treatments required to sterilize cooked ground beef inoculated with PA 3679 spores have been determined. Studies were also carried out with raw meat pack.

Table I includes data from runs PA-2 through PA-25, all of which were carried out under similar conditions as indicated. Results from these runs, are summarized in Table II. Data from Table II, plotted in Fig. 3, indicate the following for inoculated packs treated as designated:

Can size: No. 1 picnic (211 x 400)
Product: Cooked ground beef
Inoculum: 10,000 PA 3679 spores per can
Preirradiation: As indicated
Processing temperature: 230°F (NOTE: 250°F for Runs PA-2 to PA-9)
Incubation temperature: 85°F

Without irradiation, an F_0 of more than 7 was required to sterilize the canned beef; with irradiation alone, approximately 2.1 megarep were required for this purpose. Preirradiation with 1.0 megarep of gamma radiation reduced the F_0 required to produce sterility to 4.4; further reduction to an F_0 of 1.0 was obtained by preirradiation with approximately 1.9 megarep.

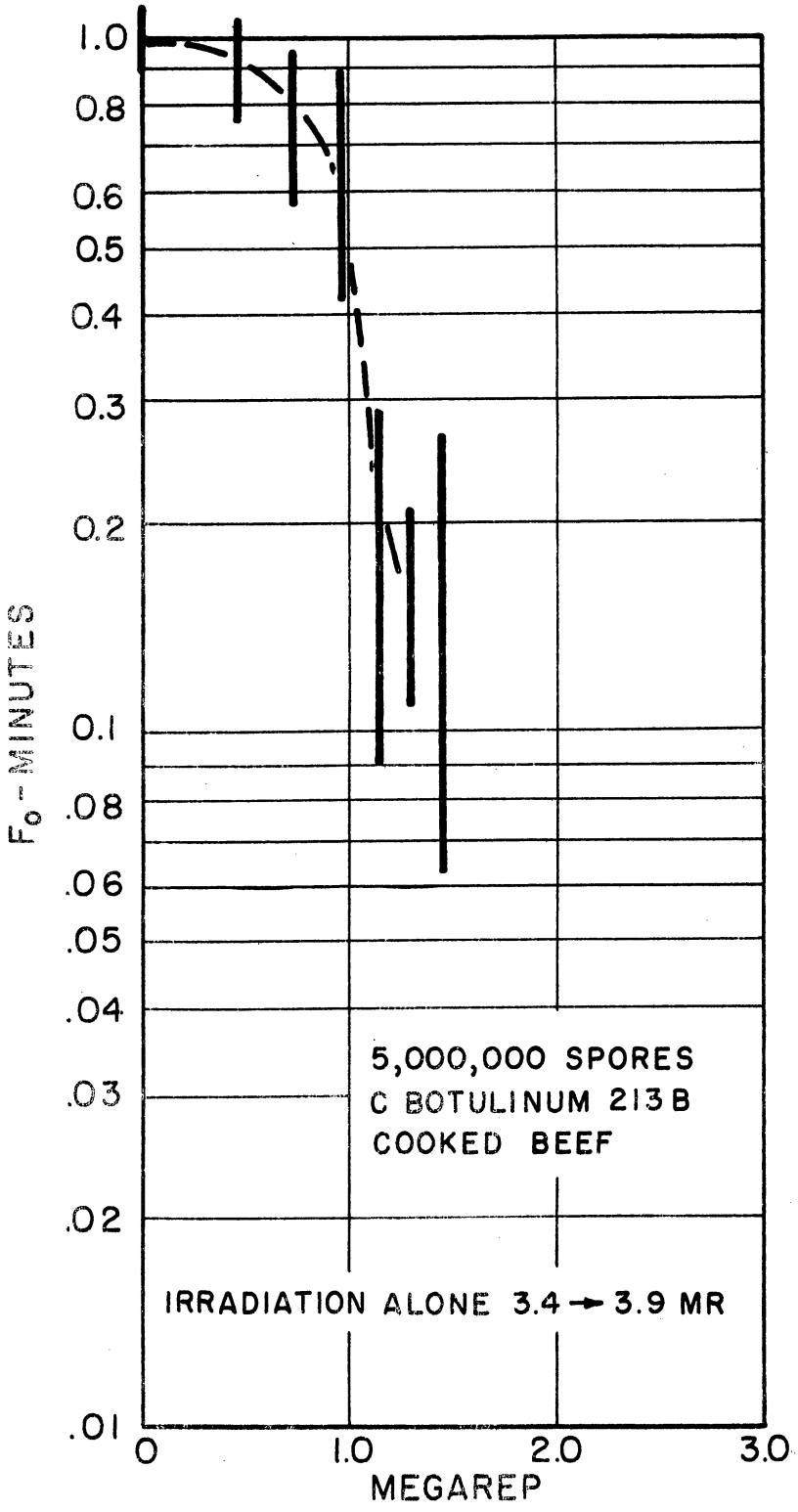

Fig. 1. F_0 required to sterilize cooked ground beef packed in No. 1 picnic tin cans, inoculated with approximately 5,000,000 C. botulinum 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat processing.

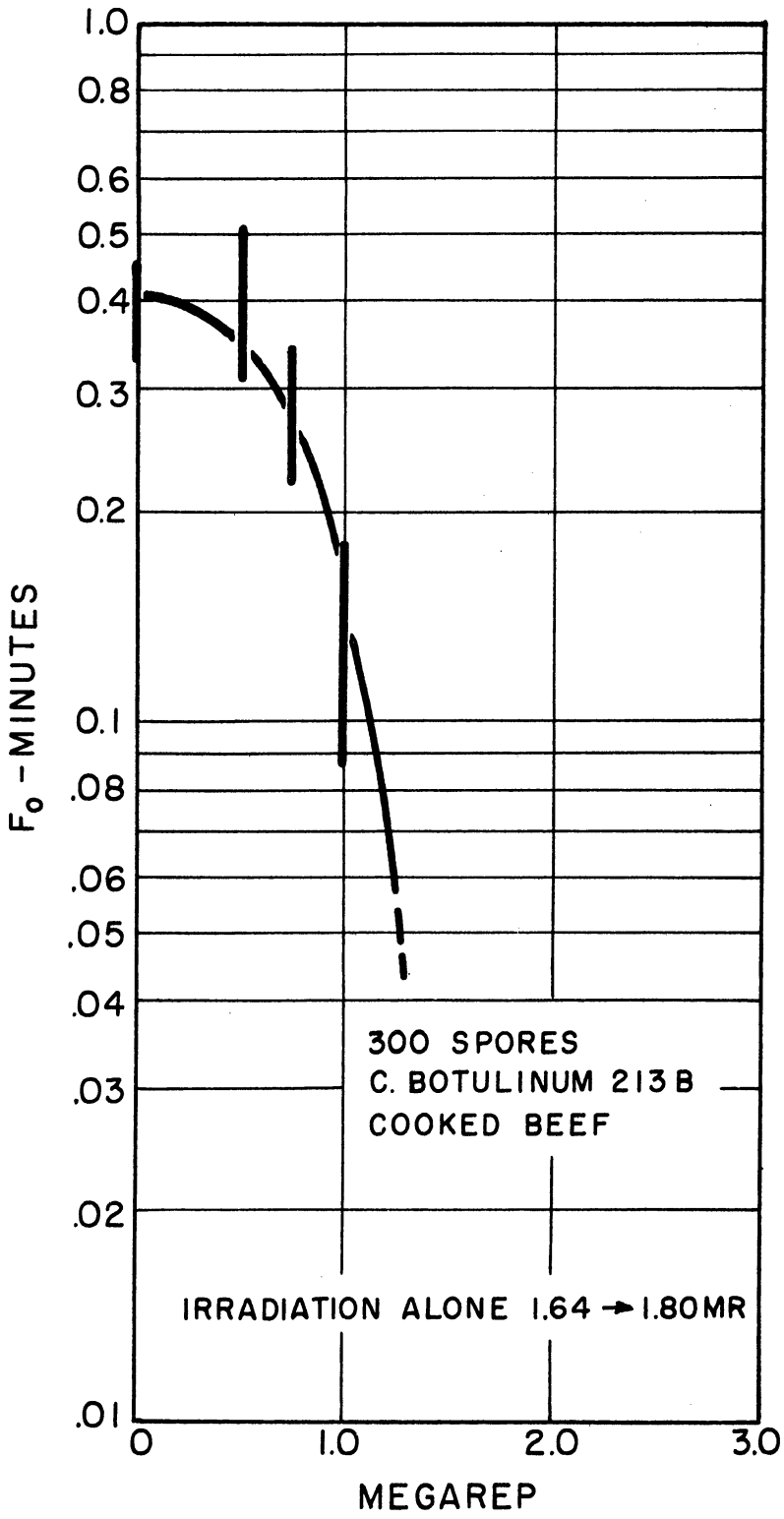

Fig. 2. F_o required to sterilize cooked ground beef packed in No. 1 picnic tin cans inoculated with approximately 300 C. botulinum 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat processing.

TABLE I. F_o Value Required to Sterilize Ground Beef in No. 1 Picnic Tin Cans, Previously Inoculated with Approximately 10,000 PA-3679 Spores per Can and Then Processed at 250°F.

Run No. PA-2—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - none
Processing Temperature - 250°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	26	3
	27	3
	28	3
	29	3
Can 1, 2.5	1	-
Can 2, 2.5	3	5
	4	4
	7	5
Can 1, 4.1	2	6
Can 2, 4.1	5	-
Can 3, 3.7	6	-
	8	-
Can 1, 1.1	9	4
Can 2, 1.1	10	4
Can 3, 1.3	11	4
	12	4
Can 1, 3.7	13	4
Can 2, 3.7	14	-
Can 3, 2.8	15	5
	16	5
Can 1, 6.2	18	-
Can 2, 3.3	19	4
	20	-
	21	-
Can 1, 8.8	22	-
Can 2, 8.8	23	-
	24	-
	25	-

Conclusion: Ground beef, packed in No. 1 picnic tin cans, and inoculated with 10,000 PA-3679 spores per can, required an F_o value between 2.8 and 8.8 for sterilization when processed at 250°F.

TABLE I (Continued)

Run No. PA-3—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 600,000 PA-3679 spores per can
Preirradiation - none
Processing Temperature - 250°F (except cans 1, 2, 3, and 4 at 230°F)
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
Inoculated Controls	I 1	3
	I 2	+*
Can 1, 2.3	1	4
Can 2, 2.3	2	4
Can 3, 2.3	3	4
	4	4
Can 1, 6.1	5	4
Can 2, 6.1	6	6
Can 3, 3.8	7	4
	8	5
Can 1, 9.4	9	9
Can 2, 8.3	10	8
Can 3, 8.7	11	6
	12	9
Can 1, 12.4	13	9
Can 2, 12.4	14	13
Can 3, 11.6	15	13
	16	16

*Date not recorded.

Conclusion: With 600,000 PA-3679 spores per can and a processing temperature of 250°F, the F_o value required for sterilization of the canned meat exceeds 12.4.

TABLE I (Continued)

Run No. PA-4—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 200 PA-3679 spores per can
Preirradiation - as indicated
Processing Temperature - not heat processed
Incubation Temperature - 85°F

Irradiation Dosage, megarep	Can No.	Days to Gas Formation
Noninoculated Controls	See Run 5	
Inoculated Controls	See Run 5	
3.550	1	-
	2	-
	3	-
	4	-
3.200	5	-
	6	-
	7	-
	8	-
1.383	9	-
	10	-
	11	6
	12	-
1.816	13	-
	14	-
	15	-
	16	-

Conclusion: Under the above conditions canned ground beef was sterilized with between 1.383 and 1.816 megarep of gamma irradiation.

TABLE I (Continued)

Run No. PA-5—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 200 PA-3679 spores per can
Preirradiation - none
Processing Temperature - 250°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	INC 1	3
	INC 2	3
	INC 3	3
	INC 4	3
Can 1, 2.7	1	-
Can 2, 2.7	2	-
	3	-
	4	-
Can 1, 3.6	5	-
Can 2, 6.8	6	-
Can 3, 8.3	8	-
	9	-
Can 1, 12.3	10	-
Can 2, 12.3	11	-
Can 3, 15.3 (disregard)	12	-
	13	-
Can 1, 9.0	14	-
Can 2, 8.0	15	-
Can 3, 8.1 (disregard)	16	-
	17	-
Can 1, 4.9	18	-
Can 2, 2.7	19	-
Can 3, 6.8	20	-
	21	-
Can 1, 4.4	23	-
Can 2, 4.4	24	-
	25	-
	26	-
Can 1, 14.1	7	-
Can 2, 12.6	22	-
	27	-
	28	-

Conclusion: Under the above conditions an F_o value less than 2.7 was needed to sterilize ground beef.

TABLE I (Continued)

Run No. PA-6—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 54,000 spores per can
Preirraidation - 1.060 megarep
Processing Temperature - 250°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
Inoculated Controls	17	4
	18	4
	19	4
	20	4
Can 1, 3.1	1	-
Can 2, 3.1	2	10
Can 3, 2.7 (disregard)	3	-
	4	-
Can 1, 3.8	5	8
Can 2, 3.5	6	-
Can 3, 4.1	7	-
	8	-
Can 1, 1.0	9	5
Can 2, 1.2	10	7
Can 3, 1.7	11	9
	12	7
Can 1, 8.6	13	-
Can 2, 6.2	14	-
Can 3, 5.5	15	-
	16	-

Conclusion: Under these conditions sterilization of canned ground beef is obtained by irradiation with 1.060 megarep of gamma rays followed by an F_o value between 3.5 and 8.6.

TABLE I (Continued)

Run No. PA-7—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 13,500 PA-3679 spores per can
Irradiation - as indicated
Processing Temperature - not processed
Incubation Temperature - 85°F

Megarep	Can No.	Days to Gas Formation
Noninoculated Controls	See Run 8	
Inoculated Controls	35	3
	36	3
	37	3
	38	3
0.853	1	4
	2	4
	3	4
	4	4
1.878	13	6
	14	-
	15	-
	16	4
1.620	31	4
	32	4
	33	4
	34	4
2.560	9	-
	10	-
	11	-
	12	-
3.330	5	-
	6	-
	7	-
	8	-

Conclusion: Irradiation-sterilization dosage for canned ground beef under these conditions lies between 1.878 and 2.560 megarep.

TABLE I (Continued)

Run No. PA-8—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 13,500 PA-3679 spores per can
Preirradiation - none
Processing Temperature - 250°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	35	4
	36	4
	37	4
	38	4
Can 1, 3.1	1	-
Can 2, 2.5	2	13
	3	6
	4	-
Can 1, 9.6	5	-
Can 2, 9.6	6	-
	7	-
	8	-
	9	-
Can 1, 5.6	10	-
Can 2, 5.6	11	-
	12	-
Can 1, 3.8	13	-
Can 2, 2.9	14	9
	15	6
	16	-
Can 1, 7.9	17	-
Can 2, 10.7	18	-
Can 3, 12.8	19	-
	20	-
Can 1, 7.0	21	-
Can 2, 7.0	22	-
	23	-
	24	-
Can 1, 11.6	25	-
Can 2, 9.2	26	-
	27	-
	28	-

Conclusion: Under these conditions canned ground beef was sterilized with heat processing at 250°F, having an F_o value between 2.5 and 5.6.

TABLE I (Continued)

Run No. PA-9—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 16,000 PA-3679 spores per can
Preirradiation - 0.853 megarep
Processing Temperature - 250°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Inoculated Controls	21	3
	23	3
	24	3
Irradiation Controls, 1.340 megarep	17	3
	18	3
	19	3
	20	3
Can 1, 2.3	1	4
Can 2, 2.3	2	5
Can 3, 0.6 (disregard)	3	4
	4	5
Can 1, 6.3	5	6
Can 2, 3.8	6	-
Can 3, 4.2	7	-
	8	7
Can 1, 0.5	9	5
Can 2, 0.5	10	4
	11	4
	12	4
Can 1, 6.5	13	-
Can 2, 9.0	14	-
Can 3, 11.7	15	-
	16	-

Conclusion: Under these conditions canned ground beef was sterilized by irradiation with 0.853 megarep of gamma irradiation followed by heat processing to an F_o value of between 3.8 and 11.7.

TABLE I (Continued) - F_o Value Required to Sterilize Ground Beef in No. 1 Picnic Tin Cans, Previously Inoculated with Approximately 10,000 PA-3679 Spores per Can and Then Processed at 230°F

Run No. PA-10—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - none
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	17	3
	18	3
	19	3
	20	3
Can 1, 6.0	1	9
Can 2, 6.0	2	11
Can 3, 5.2	3	8
	4	11
Can 1, 3.8	5	5
Can 2, 3.8	6	6
Can 3, 3.8	7	5
	8	5
Can 1, 3.0	9	6
Can 2, 3.0	10	5
Can 3, 3.0	11	5
	12	5
Can 1, 2.0	13	4
Can 2, 2.0	14	4
Can 3, 2.0	15	4
	16	4

Conclusion: Under these conditions the F_o value required to sterilize canned ground beef exceeds 6.0.

TABLE I (Continued)

Run No. PA-11—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 1.261 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	17	3
	18	3
	19	3
	20	3
Can 1, 1.0	1	5
Can 2, 0.8	2	5
Can 3, 0.4	3	5
	4	5
Can 1, 1.6	5	6
Can 2, 1.0	6	5
Can 3, 0.8	7	5
	8	6
Can 1, 2.0	9	9
Can 2, 2.0	10	6
Can 3, 3.0	11	9
	12	-
Can 1, 3.9	13	-
Can 2, 3.9	14	-
Can 3, 4.9	15	-
	16	-

Conclusion: Canned ground beef was sterilized under these conditions by 1.500 megarep of preirradiation followed by heat processing with an F_o value between 2.0 and 4.9.

TABLE I (Continued)

Run No. PA-12—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - None
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
Inoculated Controls	INOC	3
Can 1, 2.9	1	4
Can 2, 2.9	2	4
	3	3
	4	5
Can 1, 5.8	5	14
Can 2, 5.8	6	-
	7	11
	8	14
Can 1, 4.3	9	6
Can 2, 5.5	10	7
	11	6
	12	6

Conclusion: Canned ground beef requires an F_o value of more than 5.8 for sterilization under these conditions.

TABLE I (Continued)

Run No. PA-13—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 0.841 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Can 1, 4.4*	1	-
Can 2, 4.4	2	7
	3	-
	4	-
Can 1, 0.7	5	4
Can 2, 0.5	6	3
	7	4
	8	4
Can 1, 2.3	9	7
Can 2, 2.3	10	4
	11	5
	12	5
Can 1, 5.7	13	-
Can 2, 5.7	14	-
	15	-
	16	-

Conclusion: Canned ground beef was sterilized under these conditions by 0.841 megarep preirradiation followed by heat processing with an F_o value between 4.4 and 5.7.

*Same controls as used for Run 12.

TABLE I (Continued)

Run No. PA-14—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 1.420 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	17	3
	18	3
	19	3
	20	3
Can 1, 5.6	1	-
Can 2, 4.9	2	-
	3	-
	4	-
Can 1, 6.5	5	-
Can 2, 5.6	6	-
	7	-
	8	-
Can 1, 1.1	9	9
Can 2, 1.1	10	-
	11	9
	12	-
Can 1, 3.2	13	-
Can 2, 3.2	14	-
Can 3, 3.2	15	-
	16	-

Conclusion: Canned ground beef was sterilized under these conditions by 1.420 megarep of gamma irradiation followed by heat processing with an F_o value between 1.1 and 3.2.

TABLE I (Continued)

Run No. PA-15—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - None
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	17	3
	18	3
	19	3
	20	3
Can 1, 6.5	1	21
Can 2, 7.0	2	17
Can 3, 7.0	3	54
	4	13
Can 1, 8.4	5	-
Can 2, 7.3	6	-
	7	-
	8	-
Can 1, 6.0	9	8
Can 2, 6.0	10	8
Can 3, 6.0	11	7
	12	7
Can 1, 5.0	13	11
Can 2, 5.0	14	11
Can 3, 5.0	15	11
	16	8

Conclusion: Under these conditions, canned ground beef was sterilized with an F_o value between 6.5 and 8.4.

TABLE I (Continued)

Run No. PA-16—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - None
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	13	3
	14	3
	15	3
	NOC	4
Can 1, 7.1	1	133
Can 2, 7.1	2	59
Can 3, 7.1	3	47
	4	63
Can 1, 8.7	5	-
Can 2, 8.7	6	-
	7	-
	8	-
Can 1, 4.1	9	7
Can 2, 4.6	10	7
	11	7
	12	7

Conclusion: Under these conditions canned ground beef was sterilized with an F_o value between 7.1 and 8.7.

TABLE I (Concluded)

Run No. PA-17—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 0.708 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	NI 1	-
	NI 2	-
	NI 3	-
	NI 4	-
Inoculated Controls	13	3
	14	3
	15	3
	INOC	4
Can 1, 10.2	1	-
Can 2, 10.2	2	-
Can 3, 10.2	3	-
	4	-
Can 1, 5.5	5	-
Can 2, 4.9	6	-
	7	-
	8	-
Can 1, 6.9	9	-
Can 2, 6.6	10	-
	11	-
	12	-
Can 1, 3.7	13	-
Can 2, 3.1	14	17
	15	12
	16	12

Conclusion: Canned ground beef was sterilized under these conditions by 0.708 megarep irradiation followed by heat processing with an F_o value between 3.1 and 5.5.

TABLE I (Continued) - F_o Value Required to Sterilize Ground Beef in No. 1 Picnic Tin Cans, Previously Inoculated with 10,000 PA-2679 Spores per Can and Irradiated with Gamma Rays from Cobalt-60 Before Processing at 230°F.

Run No. PA-18—Can size - No. 1 picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 1.652 megarep
Processing temperature - 230°F
Incubation temperature - 85°F

F_o	Can No.	Days to Gas Formation
Inoculated Controls	1	4
	2	4
	3	4
	4	4
Can 1, 1.1	1	-
Can 2, 1.1	2	-
Can 3, 0.68	3	-
	4	-
Can 1, 1.8	5	-
Can 2, 1.8	6	-
	7	-
	8	13
Can 1, 0.16	9	5
Can 2, 0.16	10	5
Can 3, 0.10	11	6
	12	-
Can 1, 0.57	13	5
Can 2, 0.40	14	-
Can 3, 0.25	15	-
	16	5
Can 1, 0.27	17	6
Can 2, 0.27	18	-
Can 3, 0.27	19	8
	20	4

Conclusion: Under these conditions canned ground beef was not sterilized by 1.652 megarep of gamma radiation followed by heat processing to an F_o of 1.8.

TABLE I (Continued)

Run No. PA-19—Can size - No. 1 picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Irradiation only
Incubation temperature - 85°F

Megarep	Can No.	Days to Gas Formation
Inoculated Controls	1	4
	2	4
Noninoculated controls	See run PA-20	
1.560	13	4
	14	4
	15	4
	16	5
1.970	9	-
	10	7
	11	-
	12	-
2.625	1	-
	2	-
	3	-
	4	-
3.040	5	-
	6	-
	7	-
	8	-

Conclusion: Canned ground beef was sterilized under these conditions by 1.970 to 2.625 megarep of gamma radiation.

TABLE I (Continued)

Run No. PA-20—Can size - No. 1 picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 0.410 megarep
Processing temperature - 230°F
Incubation temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	See run PA-19	
Can 1, 7.9	1	-
Can 2, 7.9	2	-
	3	-
	4	-
Can 1, 4.5	5	40
Can 2, 4.5	6	-
	7	23
	8	13
Can 1, 6.1	9	6
Can 2, 6.1	10	17
Can 3, 5.7	11	-
	12	6
Can 1, 3.1	13	6
Can 2, 3.1	14	7
Can 3, 3.1	15	-
	16	5

Conclusion: Under these conditions, canned ground beef was sterilized by 0.410 megarep of gamma radiation followed by heat processing with an F_o between 5.7 and 7.9.

TABLE I (Continued)

Run No. PA-21—Can size - No. 1 picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 1.313 megarep
Processing temperature - 230°F
Incubation temperature - 85°F

F_o	Can No.	Days to Gas Formation
Controls	See run PA-19	
Can 1, 0.44	1	4
Can 2, 0.34	2	3
Can 3, 0.29	4	3
	8	3
Can 1, 6.6	5	-
Can 2, 6.6	6	-
Can 3, 6.0	3	-
	7	-
Can 1, 4.0	9	-
Can 2, 4.0	10	-
Can 3, 4.0	11	-
	12	-

Conclusion: Under these conditions, canned ground beef was sterilized by 1.313 megarep of gamma radiation followed by an F_o between 0.29 and 4.0.

TABLE I (Continued)

Run No. PA-22—Can size - No. 1 picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 1.780 megarep
Processing temperature - 230°F
Incubation temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 2.4	1	-
Can 2, 2.1	2	-
Can 3, 1.9	3	-
	4	-
Can 1, 1.5	5	-
Can 2, 0.97	6	-
Can 3, 0.78	7	8
	8	-
Can 1, 0.56	9	4
Can 2, 0.56	10	4
	11	4
	12	6
Can 1, 0.23	13	3
Can 2, 0.20	14	4
Can 3, 0.20	15	4
	16	5

Conclusion: Under these conditions, canned ground beef was sterilized by 1.780 megarep of gamma radiation followed by an F_o between 0.78 and 2.4.

TABLE I (Continued)

Run No. PA-23—Can size - No. 1 picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 1.538 megarep
Processing temperature - 230°F
Incubation temperature - 85°F

F_o	Can No.	Days to Gas Formation
Controls	See run PA-22	
Can 1, 2.3	1	4
Can 2, 2.3	2	-
Can 3, 2.3	3	-
	4	-
Can 1, 1.1	5	4
Can 2, 0.97	6	4
Can 3, 0.97	7	-
	8	-
Can 1, 0.14	9	3
Can 2, 0.14	10	3
Can 3, 0.16	11	3
	12	3
Can 1, 3.1	13	-
Can 2, 3.7	14	-
Can 3, 3.4	15	-
	16	-
Can 1, 4.2	17	-
Can 2, 4.1	18	-
Can 3, 3.8	19	-
	20	-

Conclusion: Under these conditions, canned ground beef was sterilized by 1.538 megarep of gamma radiation followed by an F_o between 2.3 and 3.7.

TABLE I (Continued)

Run No. PA-24—Can size - No. 1 picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Preirradiation - 1.985 megarep
Processing temperature - 230°F
Incubation temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 0.25	1	-
Can 2, 0.23	2	-
Can 3, 0.22	3	-
	4	-
Can 1, 0.061	5	-
Can 2, 0.055	6	4
Can 3, 0.068	7	-
	8	-
Can 1, 1.61	9	-
Can 2, 1.40	10	-
Can 3, 1.32	11	-
	12	-
Can 1, 0.63	13	-
Can 2, 0.49	14	-
Can 3, 0.41	15	-
	16	-

Conclusion: Under these conditions, canned ground beef was sterilized by 1.985 megarep of gamma radiation followed by an F_o between 0.055 and 0.25.

TABLE I (Concluded)

Run No. PA-25—Can size - No. 1 picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 10,000 PA-3679 spores per can
Irradiation only
Incubation temperature - 85°F

Megarep	Can No.	Days to Gas Formation
Controls	See run PA-24	
1.620	1	4
	4	3
	5	3
	8	3
2.160	2	-
	3	-
	6	-
	7	-
2.440	9	-
	10	-
	11	-
	12	-
	13	-
	14	-

Conclusion: Under these conditions, canned ground beef was sterilized by 1.620 to 2.160 megarep of gamma radiation.

TABLE II. Summary of Various Combined Irradiation Heat-Processing Treatments Required to Sterilize Cooked Ground Beef in No. 1 Picnic Tin Cans Previously Inoculated with 10,000 PA-3679 Spores per Can.

Run No.	Preirradiation, megarep*	F_0 Range, minute
PA-10	0	> 6.0
PA-11	1.261	2.0-4.9
PA-12	0	> 5.8
PA-13	0.841	4.4-5.7
PA-14	1.420	1.1-3.2
PA-15	0	6.5-8.4
PA-16	0	7.1-8.7
PA-17	0.708	3.1-5.5
PA-18	1.652	> 1.80
PA-19	1.970-2.625	0
PA-20	0.410	5.7-7.9
PA-21	1.313	0.29-4.0
PA-22	1.780	0.78-2.4
PA-23	1.538	2.3-3.7
PA-24	1.985	0.055-0.25
PA-25	1.620-2.160	0

*Note: corrected on basis of source recalibration, 1-7-57

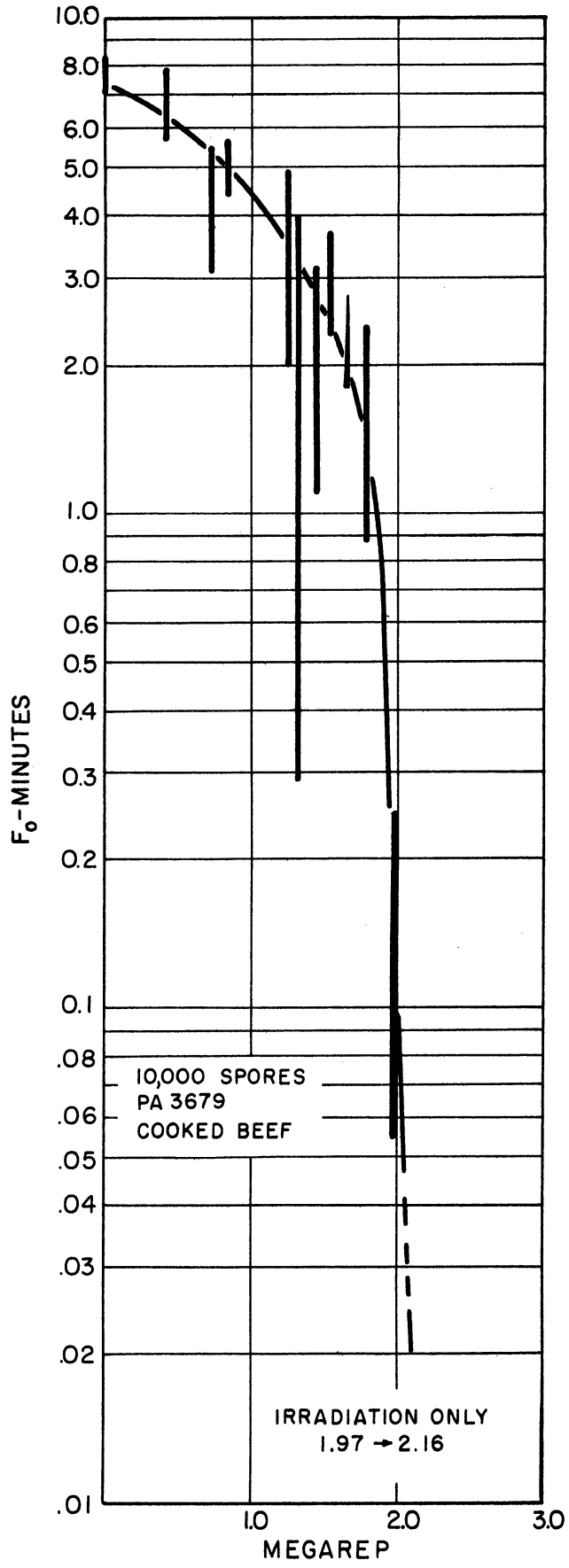

Fig. 3. F_o required to sterilize cooked ground beef packed in No. 1 picnic tin cans, inoculated with 10,000 PA 3679 spores per can, and irradiated with gamma rays from cobalt-60 before heat processing at 230°F.

Table III includes data from runs PA26 through PA-33. These runs are similar to those previously described in Table I except that only 300 PA 3679 spores were inoculated into each can. The results for these runs are summarized in Table IV and plotted in Fig. 4. They show that, under these conditions, without irradiation an F_0 of approximately 6 was required to sterilize the precooked ground beef; with irradiation alone, between 1.750 and 2.020 megarep were needed; but when irradiation preceded heat processing, those cans of cooked ground beef receiving more than 1.3 megarep were subsequently sterilized with F_0 values less than 1.0.

Table V includes data from runs RCB-1 through RCB-11 which are similar to those previously described in Table I except that the meat was packed into the cans while still in the raw condition, and each can was inoculated with approximately 5,000,000 C. botulinum 213B spores. The results for these runs are summarized in Table VI and plotted in Fig. 5. The data show that, under these conditions, sterilization was accomplished by heat processing alone with an F_0 between 1.0 and 1.1; with irradiation only, between 2.75 and 3.00 megarep were needed; but when irradiation preceded heat processing, 1.2 megarep of gamma radiation reduced the F_0 subsequently required for sterilization to approximately 0.2.

Table VII includes data from runs RPA-1 through RPA-7. These runs are similar to those tabulated in Table V except that 300 PA 3679 spores were used for the inoculum in each can of raw meat. The results for these runs are summarized in Table VIII and are plotted in Fig. 6. The data show that raw ground beef, packed in No. 1 picnic tin cans and inoculated with 300 PA 3679 spores per can, was sterilized by heat processing alone with an F_0 of approximately 6, and by irradition alone, with between 2.000 and 2.300 megarep, but when irradiation preceded heat processing, 1.3 megarep reduced the F_0 subsequently required to less than 1.0.

DISCUSSION

The spores of C. botulinum were chosen for the initial portion of this work because of their importance in food poisoning. Also, the toxin developed by their growth permitted testing of the processed cans to insure that spoilage was caused by growth of the injected spores and not by contaminants. This was useful for development of the techniques later used for PA 3679 spore packs.

The first part of the work was also carried out with cooked, sterile, ground beef. This was done for several reasons, viz:

1. Cooked meat is a classical medium for growing anaerobic bacterial spores to produce gas.

2. A sterile medium permits the usual methods of pure culture bacteriological

TABLE III. F_o Value Required to Sterilize Cooked Ground Beef in No. 1 Picnic Tin Cans, Previously Inoculated with Approximately 300 PA-3679 Spores per Can and Then Processed at 230°F

Run No. PA-26—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 0.500 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 1.97	1	5
Can 2, 1.97	2	5
Can 3, 1.97	3	4
	4	5
Can 1, 2.87	5	6
Can 2, 2.83	6	9
Can 3, 2.49	7	4
	8	45
Can 1, 7.42	9	-
Can 2, 7.43	10	-
Can 3, 6.51	11	-
	12	-
Can 1, 4.50	13	-
Can 2, 4.81	14	-
Can 3, 4.85	15	-
	16	-

Conclusion: Sterilization was accomplished with 0.500 megarep irradiation followed by an F_o between 2.5 and 4.9.

TABLE III (Continued)

Run No. PA-27—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - none
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Controls — See Run No. PA-26		
Can 1, 0.63	1	4
Can 2, 0.55	2	4
Can 3, 0.62	3	4
	4	4
Can 1, 0.36	5	3
Can 2, 0.28	6	3
Can 3, 0.29	7	3
	8	3
Can 1, 8.10	9	-
Can 2, 8.46	10	-
Can 3, 7.77	11	-
	12	-
Can 1, 1.91	13	5
Can 2, 1.76	14	5
	15	4
	16	4
Can 1, 1.02	17	4
Can 2, 0.59	18	3
	19	4
	20	4
Can 1, 5.01	21	-
Can 2, 5.01	22	-
	23	17
	24	-
Can 1, 3.14	25	7
Can 2, 3.14	26	5
Can 3, 3.14	27	6
	28	7
Can 1, 4.07	29	8
Can 2, 4.31	30	6
Can 3, 3.53	31	6
	32	5
Can 1, 7.05	33	-
Can 2, 7.05	34	-
Can 3, 7.05	35	-
	36	-

Conclusion: Sterilization was accomplished with an F_o between 5.0 and 7.1.

TABLE III (Continued)

Run No. PA-28—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 1.000 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 0.90	1	5
Can 2, 0.90	2	-
Can 3, 0.46	3	10
	4	8
Can 1, 1.8	5	-
Can 2, 1.1	6	7
	7	-
	8	-
Can 1, 3.50	9	-
Can 2, 4.68	10	-
Can 3, 4.95	11	-
	12	-
Can 1, 2.0	13	-
Can 2, 2.6	14	-
Can 3, 2.9	15	-
	16	10

Conclusion: Sterilization was accomplished with 1.000 megarep of gamma radiation followed by an F_o between 2.0 and 5.0.

TABLE III (Continued)

Run No. PA-29—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 0.750 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Controls: — See Run No. PA-28		
Can 1, 1.84	1	6
Can 2, 1.43	2	6
Can 3, 1.65	3	5
	4	5
Can 1, 2.56	5	-
Can 2, 2.56	6	10
Can 3, 2.56	7	11
	8	-
Can 1, 0.74	9	5
Can 2, 1.02	10	5
Can 3, 0.53	11	5
	12	5
Can 1, 3.48	13	-
Can 2, 3.48	14	-
Can 3, 3.68	15	-
	16	-

Conclusion: Sterilization was accomplished by 0.750 megarep of gamma radiation followed by an F_o between 2.6 and 3.7.

TABLE III (Continued)

Run No. PA-30—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 1.250 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 2.32	9	-
Can 2, 1.47	10	-
Can 3, 1.70	11	-
	12	-
Can 1, 0.38	13	5
Can 2, 0.24	14	4
Can 3, 0.12	15	4
	16	4
Can 1, 0.98	17	-
Can 2, 0.98	18	5
Can 3, 0.48	19	-
	20	-
Can 1, 1.29	21	-
Can 2, 1.29	22	-
Can 3, 1.50	23	-
	24	-

Conclusion: Sterilization was accomplished by 1.250 megarep of gamma radiation followed with an F_o between 0.48 and 1.5.

TABLE III (Continued)

Run No. PA-31—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Incubation Temperature - 85°F

Radiation Dosage (megarep)	Can No.	Days to Gas Formation
Controls — See Run No. PA-30		
1.750	1	-
	2	5
	3	-
	4	5
2.160	17	-
	18	-
	19	-
	20	-
1.250	9	3
	15	3
	12	4
	13	3
1.390	10	5
	11	4
	14	5
	16	4
2.020	5	-
	6	-
	7	-
	8	-

Conclusion: Sterilization was accomplished with between 1.750 and 2.020 megarep of gamma radiation.

TABLE III (Continued)

Run No. PA-32—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 0.250 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 3.28	1	6
Can 2, 3.49	2	5
	3	6
	4	5
Can 1, 4.83	5	7
Can 2, 4.83	6	7
Can 3, 4.52	7	11
	8	9
Can 1, 6.93	9	-
Can 2, 6.61	10	-
Can 3, 6.66	11	-
	12	-
Can 1, 7.65	13	-
Can 2, 8.09	14	-
Can 3, 8.09	15	-
	16	-

Conclusion: Sterilization was accomplished with 0.250 megarep of gamma radiation followed by an F_o between 4.5 and 6.9.

TABLE III (Concluded)

Run No. PA-33—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 1.350 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Can 1, 0.31	1	5
Can 2, 0.31	2	-
	3	-
	4	7
Can 1, 1.46	5	-
Can 2, 1.46	6	-
	7	-
	8	-
Can 1, 0.77	9	7
Can 2, 1.13	10	-
Can 3, 1.13	11	-
	12	-
Can 1, 0.58	13	-
Can 2, 0.42	14	-
Can 3, 0.54	15	-
	16	5

Conclusion: Sterilization was accomplished by 1.350 megarep of gamma radiation followed with an F_o between 0.77 and 1.5.

TABLE IV - Summary of Various Combined Irradiation—Heat-Processing Treatments Required to Sterilize Cooked Ground Beef in No. 1 Picnic Tin Cans Previously Inoculated with 300 PA-3679 Spores per Can.

Run No.	Preirradiation, megarep	F_o Range, minute
PA-26	0.500	2.5-4.9
PA-27	none	5.0-7.1
PA-28	1.000	2.0-5.0
PA-29	0.750	2.6-3.7
PA-30	1.250	0.48-1.5
PA-31	1.750-2.020	none
PA-32	0.250	4.5-6.9
PA-33	1.350	0.77-1.5

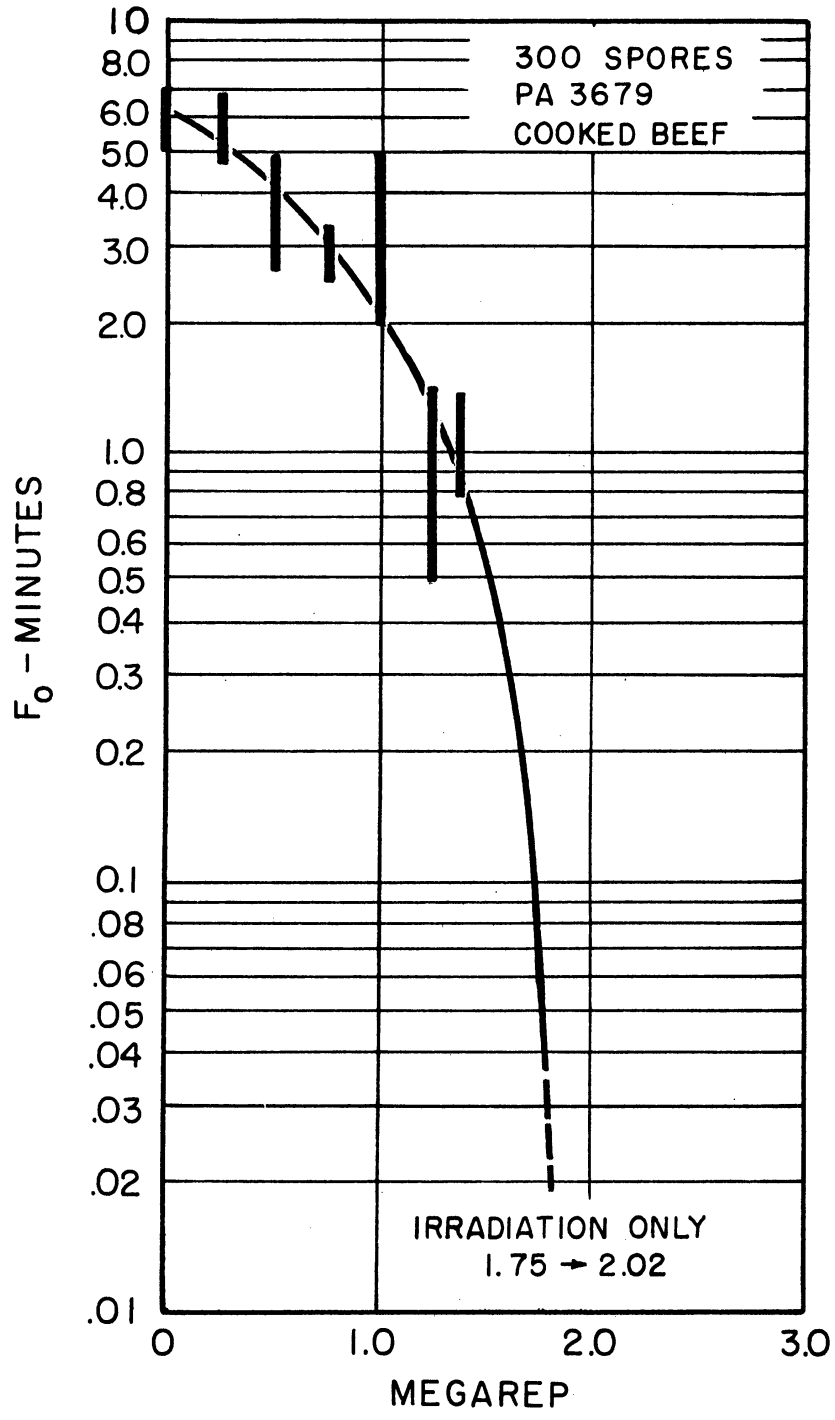

Fig. 4. F_o required to sterilize cooked ground beef packed in No. 1 picnic tin cans, inoculated with 300 PA 3679 spores per can, and irradiated with gamma rays from cobalt-60 before heat processing at 230°F.

TABLE V. F_o Values Required to Sterilize Raw Ground Beef in No. 1 Picnic Tin Cans, Previously Inoculated with 5,000,000 C. botulinum 213B Spores per Can and Then Processed at 230°F.

Run No. RCB-1—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 2,500,000 C. botulinum 213B spores per can
Preirradiation - None
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Can 1, 0.25	1	8
Can 2, 0.19	2	7
	3	7
	4	8
Can 1, 0.74	5	9
Can 2, 0.74	6	11
Can 3, 0.74	7	-
	8	10

Conclusion: Under these conditions, raw ground beef required an F_o in excess of 0.74 for sterilization.

TABLE V (Continued)

Run No. RCB-2—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 2,500,000 C. botulinum 213B spores per can
Preirradiation - None
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Can 1, 0.58	1	4
Can 2, 0.59	2	4
Can 3, 0.61	3	4
	4	4
Can 1, 0.76	5	4
Can 2, 0.76	6	4
Can 3, 0.76	7	4
	8	4
Can 1, 1.13	9	7
Can 2, 0.93	10	7
	11	7
	12	7
Can 1, 2.08	13	-
Can 2, 2.08	14	-
Can 3, 2.08	15	-
	16	-
Can 1, 1.49	17	-
Can 2, 1.49	18	-
Can 3, 1.49	19	-
	20	-

Conclusion: Under these conditions, raw ground beef was sterilized with an F_o between 0.93 and 1.5.

TABLE V (Continued)

Run No. RCB-3—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - 1.400 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Inoculated Control	17	1
	18	1
Irradiated and Inoculated	27	4
	28	5
No Heat Process	29	5
1.4 Megarep	30	4
Can 1, 0.13	1	4
Can 2, 0.16	2	-
	3	6
	4	-
Can 1, 0.34	5	-
Can 2, 0.29	6	-
Can 3, 0.29	7	-
	8	-
Can 1, 0.09	9	4
Can 2, 0.06	10	6
Can 3, 0.06	11	4
	12	4
Can 1, 0.22	13	-
Can 2, 0.22	14	-
Can 3, 0.28	15	-
	16	-

Conclusion: Under these conditions, raw ground beef was sterilized by 1.400 megarep preirradiation plus an F_o between 0.13 and 0.28.

TABLE V (Continued)

Run No. RCB-4—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - 2.000 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Inoculated and Irradiated	25	-
No Heat Process	26	-
2.130 Megarap	27	-
	28	-
Inoculated and Nonirradiated	29	-
Heat-processed	30	-
	31	-
Can 1, 1.03	32	-
Can 2, 1.03	--	-
Can 3, 1.03	--	-
Inoculated and Nonirradiated	37	-
Heat-processed	38	-
	39	-
Can 1, 1.67	40	-
Can 2, 1.67		
Can 3		
Noninoculated and Nonirradiated	1	-
	2	-
Can 1, 0.17	3	-
Can 2, 0.17	4	11
Can 3, 0.20		
Noninoculated	11	-
No heat Process	5	-
2.000 Megarep	6	-
Can 1, 0.033	17	-
Can 2, 0.033	18	-
Can 3, 0.033	19	-
	20	-
Can 1, 0.28	21	-
Can 2, 0.28	22	-
	23	-
	24	-

TABLE V (Continued)

Run No. RCB-4 (Concluded)

F_o	Can No.	Days to Gas Formation
Can 1, 0.05	13	-
Can 2, 0.04	14	-
Can 3, 0.04	15	-
	16	-
Can 1, 0.12	41	-
Can 2, 0.12	42	-
	43	-

Conclusion: 1) Under these conditions, raw ground beef was sterilized by 2.000 megarep preirradiation plus an F_o less than 0.033. 2) An F_o of 0.20 was not quite enough to sterilize the noninoculated raw meat.

TABLE V (Continued)

Run No. RCB-6—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - 0.600 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Inoculated	1	-
Not irradiated	2	-
Can 1, 1.84	3	-
Can 2, 1.75	4	-
Can 3, 1.75		
Inoculated	5	-
Not irradiated	6	-
Can 1, 0.99	7	-
Can 2, 0.99	8	-
Can 3, 0.99		
Noninoculated	NI-5	8
Nonirradiated	NI-6	-
Can 1, 0.23	NI-7	62
Can 2, 0.23	NI-8	-
Can 3, 0.26		
Inoculated	24	3
Irradiated only	28	3
0.600 megarep		
Can 1, 0.59	9	-
Can 2, 0.59	10	-
Can 3, 0.59	13	-
	14	-
Can 1, 0.21	21	4
Can 2, 0.21	22	4
Can 3, 0.18	26	4
	27	5
Can 1, 1.62	11	-
Can 2, 1.60	12	-
Can 3, 1.60	16	-
	17	-

TABLE V (Continued)

Run No. RCB-6 (Concluded)

F_o	Can No.	Days to Gas Formation
Can 1, 0.94	25	-
Can 2, 0.94	29	-
Can 3, 0.94	30	-
	34	-
Can 1, 0.38	15	-
Can 2, 0.38	18	-
Can 3, 0.37	19	-
	20	7

Conclusion: Under these conditions, raw ground beef was sterilized by 0.600 megarep preirradiation plus an F_o between 0.37 and 0.59.

TABLE V (Continued)

Run No. RCB-7—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - 1.000 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Can 1, 0.33	13	-
Can 2, 0.33	14	-
Can 3, 0.33	15	-
	16	-
Can 1, 0.10	9	-
Can 2, 0.10	10	7
Can 3, 0.10	11	8
	12	6
Can 1, 0.69	17	-
Can 2, 0.66	18	-
Can 3, 0.66	19	-
	20	-
Can 1, 0.16	21	-
Can 2, 0.13	22	-
Can 3, 0.13	27	-
	28	-
Can 1, 1.25	29	-
Can 2, 1.25	30	-
	35	-
	36	-
Can 1, 0.14	5	8
Can 2, 0.14	6	-
Can 3, 0.14	7	6
	8	6
Inoculated	37	7
Nonirradiated	38	8
Canl, 0.88	39	8
Can 2, 0.88	40	8
Inoculated	1	7
Nonirradiated	2	7
Can 1, 0.213	3	7
Can 2, 0.213	4	-

TABLE V (Continued)

Run No. RCB-7 (Concluded)

F_o	Can No.	Days to Gas Formation
Inoculated	23	-
Nonirradiated	24	16
Can 1, 0.761	31	-
	32	-
Noninoculated	NI	7
Nonirradiated	NI	47
Can 1, 0.17	NI	-
Can 2, 0.17	NI	-
Can 3, 0.17		

Conclusion: Under these conditions, raw ground beef was sterilized by 1.000 megarep followed by an F_o between 0.14 and 0.33.

TABLE V (Continued)

Run No. RCB-8—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - 1.700 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated	NI	-
Nonirradiated	NI	-
Can 1, 0.21	NI	-
Can 2, 0.21	NI	-
Can 3, 0.19		
Inoculated	25	-
Nonirradiated	26	-
Can 1, 1.20	33	-
Can 2, 1.20	34	-
Can 3, 1.20		
Can 1, 0.02	9	7
Can 2, 0.02	10	7
Can 3, 0.02	14	7
	17	7
Can 1, 0.03	13	6
Can 2, 0.03	15	-
	16	7
	18	6
Can 1, 1.54	45	-
Can 2, 1.54	46	-
Can 3, 1.54	47	-
	48	-
Can 1, 0.025	41	-
Can 2, 0.025	42	-
Can 3, 0.025	43	-
	44	-

Conclusion: Under these conditions, raw ground beef was sterilized by 1.700 megarep followed with an F_o between 0.03 and 1.5.
Note: Cans 41 through 48 were run with a different batch of meat and 3 weeks later.

TABLE V (Continued)

Run No. RCB-9—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - 0.700 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Can 1, 1.09	1	-
Can 2, 1.09	2	-
Can 3, 1.09	3	-
	4	-
Can 1, 0.31	11	10
Can 2, 0.31	12	-
Can 3, 0.31	21	15
	22	-
Can 1, 0.43	24	-
Can 2, 0.43	26	-
Can 3, 0.39	27	-
	28	8
Can 1, 0.15	23	5
Can 2, 0.15	25	5
	19	5
	20	5

Conclusion: Under these conditions, raw ground beef was sterilized by 0.700 megarep followed by an F_o between 0.39 and 1.1

TABLE V (Continued)

Run No. RCB-10—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - None
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated	NI	-
Nonirradiated	NI	-
Can 1, 0.12	NI	-
Can 2, 0.12	NI	-
Can 3, 0.12		
Can 1, 0.53	1	7
Can 2, 0.53	2	7
Can 3, 0.55	3	7
	4	7
Can 1, 1.02	5	-
Can 2, 1.02	6	-
Can 3, 1.09	7	12
	8	-
Can 1, 0.79	9	21
Can 2, 0.72	10	12
Can 3, 0.75	11	12
	12	12
Can 1, 1.12	13	-
Can 2, 1.12	14	-
Can 3, 1.12	15	-
	16	-

Conclusion: Under this condition, raw ground beef was sterilized by an F_o between 1.0 and 1.1.

TABLE V (Concluded)

Run No. RCB-11—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - as indicated
Processing Temperature -
Incubation Temperature - 85°F

Megarep	Can No.	Days to Gas Formation
3.000	1	-
	2	-
	3	-
	4	-
3.500	5	-
	6	-
	7	-
	8	-
2.250	9	4
	10	4
	11	4
	12	4
2.750	13	-
	14	4
	15	-
	16	-

Conclusion: Under these conditions, raw ground beef was sterilized by between 2.75 and 3.000 megarep of gamma radiation.

TABLE VI - Summary of Various Combined Irradiation—Heat Processing Treatments Required to Sterilize Raw Ground Beef in No. 1 Picnic Tin Cans Previously Inoculated with 5,000,000 C. botulinum 213B Spores per Can.

Run No.	Preirradiation, megarep	F_o Range, minutes
RCB-1	none	> 0.74
RCB-2	none	0.93-1.5
RCB-3	1.400	0.13-0.28
RCB-4	2.000	< 0.033
RCB-6	0.600	0.37-0.59
RCB-7	1.000	0.14-0.33
RCB-8	1.700	0.03-1.5
RCB-9	0.700	0.39-1.1
RCB-10	none	1.0-1.1
RCB-11	2.750-3.000	none

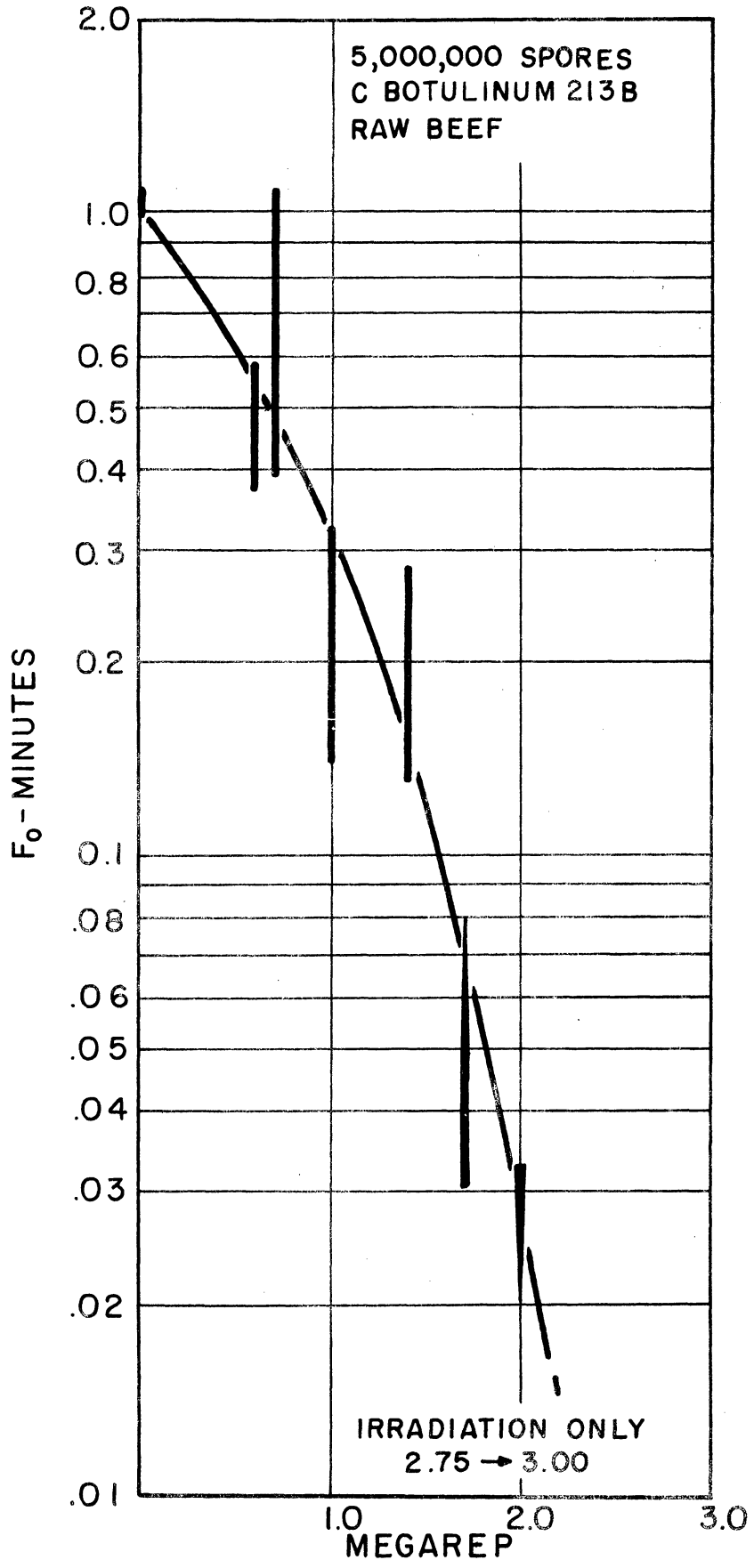

Fig. 5. F_o required to sterilize raw ground beef packed in No. 1 picnic tin cans, inoculated with 5,000,000 C. botulinum 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat processing at 230°F.

TABLE VII. F_o Value Required to Sterilize Raw Ground Beef in No. 1 Picnic Tin Cans, Previously Inoculated with Approximately 300 PA-3679 Spores per Can and Then Processed at 230°F.

Run No. RPA-1—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 300 PA-3679 spores
Preirradiation - None
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Can 1, 5.25	1	33
Can 2, 5.25	2	9
Can 3, 5.25	3	-
	4	-
Can 1, 7.84	5	-
Can 2, 7.84	6	-
Can 3, 7.84	7	-
	8	-

Conclusion: Under these conditions, raw ground beef was sterilized by an F_o between 5.3 and 7.8.

TABLE VII (Continued)

Run No. RPA-3—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 0.400 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Not irradiated	1	6
Can 1, 4.35	2	-
Can 2, 4.35	3	5
Can 3, 4.35	4	4
Not irradiated	5	-
Can 1, 6.63	6	-
Can 2, 6.63	7	-
Can 3, 6.63	8	-
Can 1, 6.54	9	-
Can 2, 6.54	10	-
Can 3, 6.54	11	-
	12	-
Can 1, 4.45	13	-
Can 2, 4.45	14	-
Can 3, 4.45	15	-
	16	-
Can 1, 3.06	17	-
Can 2, 3.06	18	-
Can 3, 3.06	19	-
	20	7
Can 1, 2.10	21	5
Can 2, 2.10	22	4
Can 3, 2.10	23	5
	24	5

Conclusion: Under these conditions, raw ground beef was sterilized by 0.400 megarep followed by an F_o between 3.1 and 4.5; without irradiation, an F_o between 4.4 and 6.6 was required.

TABLE VII (Continued)

Run No. RPA-4—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 300 PA-3679 spores per can
Irradiation - As indicated
Processing Temperature -
Incubation Temperature - 85°F

Megarep	Can No.	Days to Gas Formation
2.000	1	4
	2	-
	3	-
	4	-
2.300	5	-
	6	-
	7	-
	8	-
1.400	9	discarded
	10	discarded
	11	discarded
	12	discarded
1.400	13	discarded
	14	discarded
	15	discarded
	16	discarded

Discussion: This was an unusual run for a number of reasons, viz.:

Meat was ground on June 11 and received that day. Due to changes in personnel at this time, it was placed in the refrigerator and not packed until the morning of June 13. On June 14, the first 8 cans were irradiated while the second 8 were kept in the refrigerator. Two refrigerators were used; both kept the meat at 40 to 50°F.

During irradiation, the cans were cold, being packed with dry ice, which, however, evaporated before termination of irradiation.

Cans 1, 2, 3, and 5 were slightly "soft" when removed from the center-well of the irradiation source on the morning of June 14. However, cans 9 through 16 were soft at this time, which was the time when they were placed in the center well. By the morning of June 15, cans 9 through 16 were slightly swollen and when warmed to incubator temperature, they were soft springers. These phenomena have been noted in lesser degree before with raw meat.

These results were interpreted to indicate growth of psychrophile bacteria, before irradiation, with gas production. By packing the meat as

TABLE VII (Continued)

Run No. RPA-4 (Concluded)

soon as it was ground, and then storing the 8 cans awaiting irradiation at 37°F, this problem has been completely circumvented for runs RPA-5 through RPA-7.

This is important when considering possible commercial use of the combined irradiation procedure, and will be considered in the report under "Discussion."

Conclusion: Under these conditions, between 2.000 and 2.300 megarep were required to sterilize the raw ground beef.

TABLE VII (Continued)

Run No. RPA-5—Can Size - No. Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 300 PA-3679 spores per can
Irradiation - 0.800 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated	1	4
Nonirradiated	2	5
Can 1, 0.24	3	4
Can 2, 0.24		
Can 1, 1.47	4	6
Can 2, 1.47	5	5
Can 3, 1.47	6	4
	7	-
Can 1, 2.14	8	4
Can 2, 2.14	9	4
	10	4
	11	-
Can 1, 2.86	12	11
Can 2, 2.86	13	-
Can 3, 2.86	14	-
	15	-
Can 1, 4.13	16	-
Can 2, 4.13	17	-
	18	-
	19	-

Conclusion: Under these conditions, raw ground beef was sterilized by 0.800 megarep followed with an F_o between 2.9 and 4.1.

TABLE VII (Continued)

Run No. RPA-6—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 300 PA-3679 spores per can
Irradiation - 1.200 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated	1	-
Nonirradiated	2	-
Can 1, 0.38	3	3
Can 2, 0.38	4	4
Can 3, 0.38		
Can 1, 0.54	5	6
Can 2, 0.54	6	3
	7	4
	8	-
Can 1, 0.86	9	4
Can 2, 0.86	10	-
	11	-
	12	5
Can 1, 1.12	13	5
Can 2, 1.12	14	5
Can 3, 1.12	15	-
	16	6
Can 1, 1.66	17	-
Can 2, 1.66	18	-
Can 3, 1.66	19	-
	20	-

Conclusion: Under these conditions, raw ground beef was sterilized by 1.200 megarep followed by an F_o between 1.1 and 1.7.

TABLE VII (Concluded)

Run No. RPA-7—Can Size - No. 1 Picnic (211 x 400)
Product - Raw Ground Beef
Inoculum - 300 PA-3679 spores per can
Irradiation - 1.500 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated	1	-
Nonirradiated	2	3
Can 1, 0.33	3	4
Can 2, 0.33	4	4
Can 3, 0.33		
Can 1, 0.71	5	-
Can 2, 0.71	6	-
Can 3, 0.71	7	-
	8	-
Can 1, 0.42	9	4
Can 2, 0.42	10	4
Can 3, 0.42	11	-
	12	7
Can 1, 0.23	13	-
Can 2, 0.23	14	-
Can 3,0.23	15	-
	16	-
Can 1, 0.13	17	-
Can 2, 0.13	18	4
Can 3, 0.13	19	5
	20	-

Conclusion: Under these conditions, raw ground beef was sterilized by 1.500 megarep followed with an F_o between 0.42 and 0.71.

TABLE VIII - Summary of Various Combined Irradiation—Heat Processing Treatments Required to Sterilize Raw Ground Beef in No. 1 Picnic Tin Cans Previously Inoculated with 300 PA 3679 Spores per Can.

Run No.	Preirradiation, megarep	F_o Range, minutes
RPA-1	none	5.3-7.8
RPA-3	0.400	3.1-4.5
RPA-3	none	4.4-6.6
RPA-4	2.000-2.300	none
RPA-5	0.800	2.9-4.1
RPA-6	1.200	1.1-1.7
RPA-7	1.500	0.42-0.71

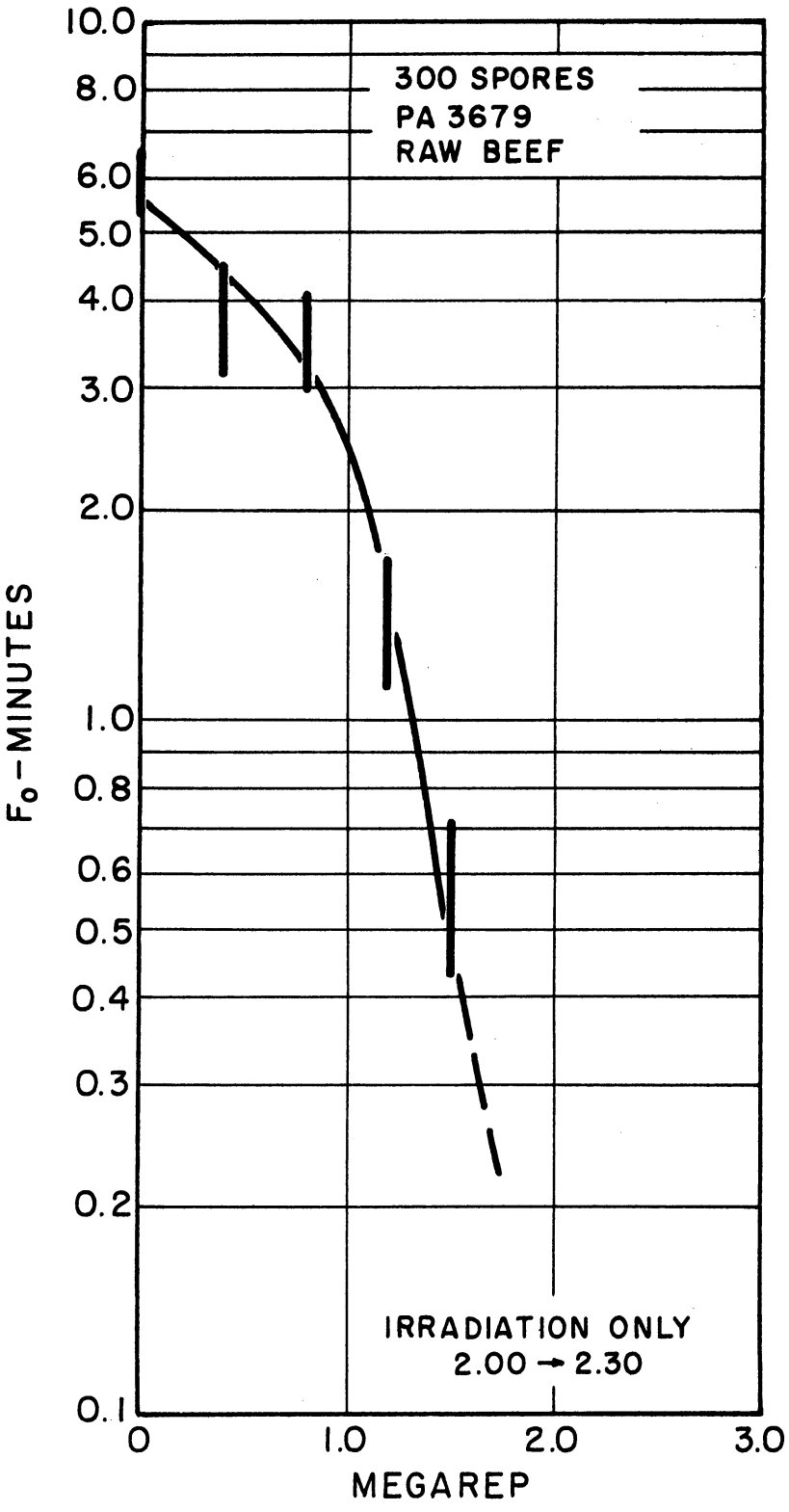

Fig. 6. F_o required to sterilize raw ground beef packed in No. 1 picnic tin cans, inoculated with 300 PA 3679 spores per can and irradiated with gamma rays from cobalt-60 before heat processing at 230°F.

techniques to be used for interpretation of the results.

3. Experience was already at hand for dealing with irradiations of cooked, canned ground beef.[1]

It was realized, however, that commercial adaptation of any process developed would likely involve preirradiation of raw meat. Since it had already been established[3] that chemicals could alter the lethal character of ionizing radiations, it was desirable to learn whether the substances present in raw meat could also act in this manner. The results indicated that, for raw beef at least, there is no difference between combined irradiations—heat processing schedules developed for precooked and for raw meat.

It was necessary to develop techniques for the heat-processing phase of this work that would produce consistent results. The method described under "Materials and Methods" provided reasonable certainty in the reported results, but ultimately any process used commercially should be tested with a larger number of cans.

Data presented in the 1956 annual report show that irradiation sensitization of spores to heat occurred only if temperatures above 85°C were used. For this reason it was possible to preheat the cans to 180°F in the autoclave before heat processing without affecting the results. The preheating permitted the development of much more uniform F_0 values among the cans being processed than when they were processed with lower starting temperatures.

It will be noted from the results with C. botulinum 213B spores that the difference between 5,000,000 and 300 spores per can did not appreciably change the combined processing treatment required. On the other hand, where either of these treatments was used alone, spore concentration was an extremely significant factor.[1,5] Actually more difference was observed between 10,000 and 300 PA 3679 spores than between 5,000,000 and 300 C. botulinum 213B spores in this regard.

Some interesting considerations are developing from this project. Sterilization of foods with ionizing radiations alone will likely require very high dosages because C. botulinum spores are very resistant to radiation. This contrasts with their relatively low heat resistance. Combined irradiation—heat processing of canned foods restores the desirable processing resistance-relationship between C. botulinum and PA 3679 spores, viz.: the latter are more resistant to combined processing than the former. Therefore, when a combined processing treatment is designed to sterilize canned meat containing PA 3679 spores, it will provide an inherent safety factor insofar as C. botulinum spores are concerned.

A question developed concerning the finding of botulinus toxin in raw meat. Part of the routine testing involves establishment of the presence

of botulinus toxin in cans of meat that develop gas upon incubation following insufficient processing when C. botulinum spores are being used for the inoculum. We have observed that botulinus toxin is often difficult to demonstrate in raw meat under these conditions. A few preliminary tests were therefore made to determine whether or not botulinus toxin developed in canned raw meat that had been previously sterilized with gamma radiation and then inoculated with C. botulinum spores. Similar results were obtained. Some of these cans of meat contained no demonstrable toxin, others contained a small amount as determined by mouse inoculation tests. Parallel, control cans of cooked meat developed large amounts of botulinus toxin. It is possible that botulinus toxin does not develop in raw meat, but more likely that it forms and then disappears. This observation should be investigated in some detail as a separate project since, if the observation is substantiated, it has considerable theoretical and practical significance. It is of interest in this connection that we cannot find any published instance of botulism poisoning resulting from raw meat, this in spite of the probable opportunity for C. botulinum organizms to grow in improperly refrigerated raw meat.

Besides the previously observed[2,4] synergistic action of gamma radiation and heat for killing anaerobic bacterial spores, other considerations indicate the desirability of a combined irradiation—heat processing treatment for sterilizing canned foods. For example, the dosage of 3.0 megarep that is often suggested for sterilizing foods will not completely inactivate enzymes, pathogenic viruses, certain micrococci, or botulinus toxin. On the other hand, viruses, micrococci, botulinus toxin, and all but a few enzymes are quickly destroyed by moist heat at 212°F. Since all these should be inactive in canned foods, a combined irradiation—heat processing treatment that is designed to kill PA 3679 spores should produce safe canned foods because any such process would involve heating the food to temperatures of 230°F or above; and, as has been shown in this report, PA 3679 spores are more resistant to combined irradiation—heat processing than C. botulinum spores.

PHASE II

COMPARISON OF Z VALUE OF IRRADIATED AND NONIRRADIATED PA 3679 SPORES

In order that F_o calculations can be intelligently carried out, it is necessary that the Z value of the spores of test bacteria be known.

There are many techniques for carrying out Z-value determinations. In general they are of two varieties, end-point analysis and decimal reduction determinations. We have previously reported[1] preliminary Z-value determinations based on end-point analysis. This time we used the survivor-curve technique, using the thermoresistometer at the American Can Co. Research Laboratories in Barrington, Illinois.

The PA 3679 spores used for this study were taken from the same stock supply as those used for the work discussed in Phase I of this report. A portion of these were irradiated with 0.500 megarep in the large cobalt-60 gamma radiation source at The University of Michigan. They were suspended in distilled water during storage and irradiation, and were refrigerated at all times.

The spore suspensions were pipetted into each of three cups of the thermoresistometer in 0.01 ml portions. These were thermally processed at the temperatures indicated, and then dropped into 0.99-ml of distilled water. The three 1.00-ml samples were then pooled and titrated as previously described. The titrations and calculated surviror data are shown in Table IX. The times required to kill 99% of the spores at the temperatures indicated are listed in Table X. The Z-value curves are plotted in Fig. 7.

It will be noted that the Z-value lines for irradiated and for nonirradiated PA 3679 spores have the same slope; this slope corresponds to a Z value of 22.3. The previous work using end-point analysis[6] developed a Z value of 23 for irradiated, and 21.3 for nonirradiated PA 3679 spores suspended in M/15 phosphate buffer at pH 7.0.

The data for this phase of the work are based on one series of experiments, however, so the results must still be considered preliminary. Nevertheless, two experiments using different techniques indicate no significant difference between the Z values of irradiated and nonirradiated PA 3679 spores.

ACKNOWLEDGMENT

We wish to express our sincere appreciation to the American Can Co. for permission to use their thermoresistometer for this work. In particular, we wish to thank Dr. E. Wheaton for his advice and assistance.

TABLE IX - Effect of Processing at Various Temperatures on PA 3679 Spores Suspended in Distilled Water Using the Thermoresistometer

Time	Number of Spores	% Survivors	Log % Survivors
110°C - Nonirradiated			
0	59,000	100	2.000
10	550	0.93	-0.031
30	7	0.01185	-1.926
50	0	---	---
110°C - Irradiated			
0	69,000	100	2.000
10	20	0.029	-1.537
30	---	---	---
50	---	---	---
115°C - Nonirradiated			
0	59,000	100	2.000
2.5	500	0.847	-0.072
5.0	192	0.326	-0.486
10.0	8	0.01358	-1.867
15.0	4	0.00678	-2.168
115°C - Irradiated			
0	69,000	100	2.000
2.5	64	0.0926	-1.033
5.0	4	0.0058	-2.237
10.0	---	---	---
15.0	---	---	---
117.5°C - Nonirradiated			
0	59,000	100	2.000
3.0	79	0.134	-0.873
5.0	22	0.0373	-1.427
10.0	---	---	---
117.5°C - Irradiated			
0	69,000	100	2.000
3.0	2	0.0029	-2.538
5.0	---	---	---

TABLE IX (Concluded)

Time	Number of Spores	% Survivors	Log % Survivors
120°C - Nonirradiated			
0	59,000	100	2.000
1.0	105	0.178	-0.749
2.0	25	0.0425	-1.370
4.0	---	---	---
120°C - Irradiated			
0	69,000	100	2.000
1.0	12	0.0174	-1.785
2.0	---	---	---
130°C - Nonirradiated			
0	59,000	100	2.000
0.2	61	0.103	-0.986
0.4	---	---	---
130°C - Irradiated			
0	69,000	100	2.000
0.2	4	0.0057	-2.244
0.4	---	---	---

TABLE X - Time Required to Kill 99% of the Nonirradiated and Irradiated PA 3679 Spores at the Temperature Indicated

Temperature, °C	Time to Kill 99%	
	Nonirradiated	Irradiated (0.500 megarep)
110	9	5.6
115	2.7	1.8
117.5	2.1	1.3
120	0.7	0.5
130	0.2	0.13

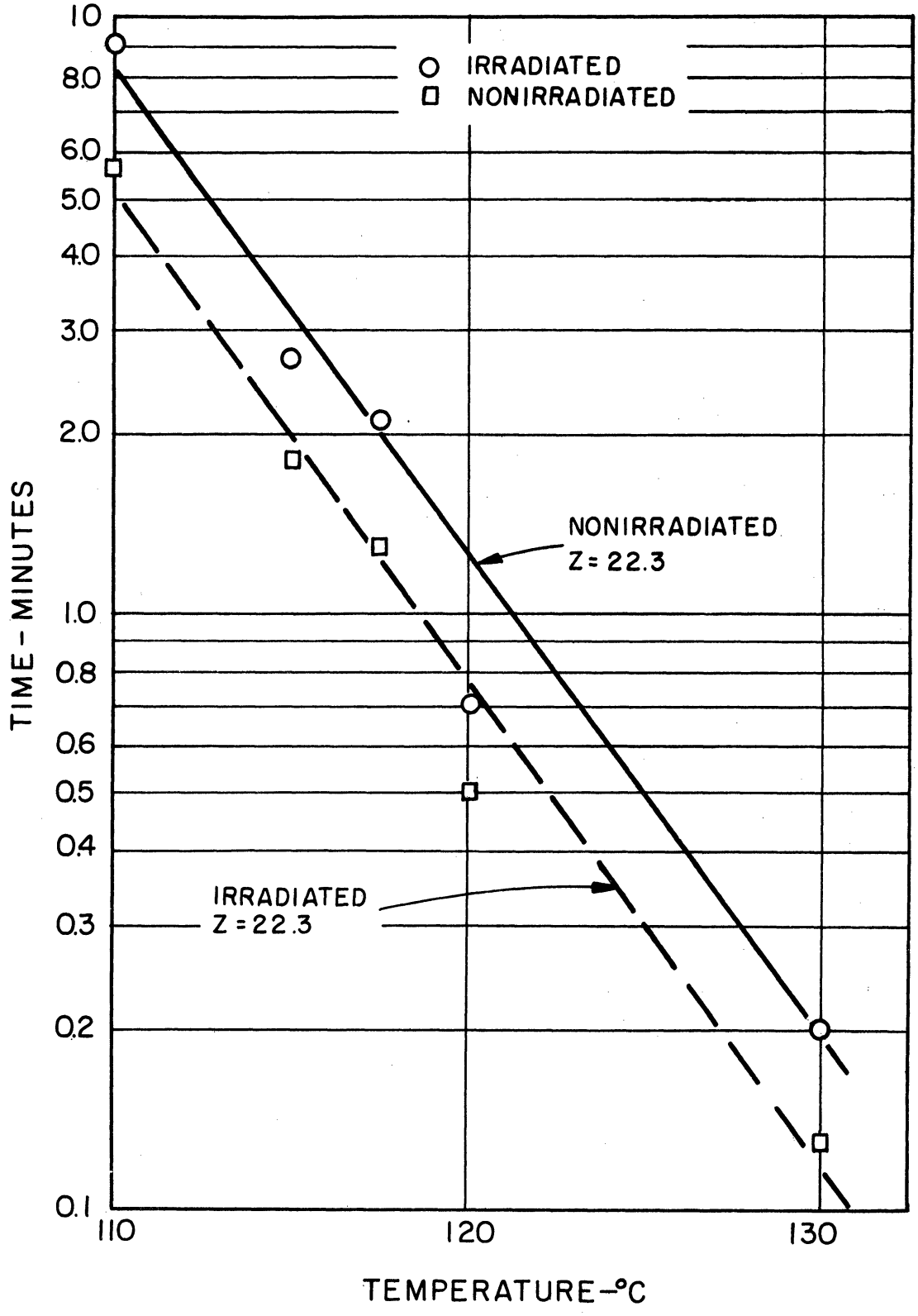

Fig. 7. Thermal death time curves for nonirradiated and irradiated PA 3679 spores suspended in distilled water.

PHASE III

THE EFFECT OF CATALASE ON THE LETHALITY OF Co-60 GAMMA RADIATION FOR CERTAIN ANAEROBIC BACTERIAL SPORES

SUMMARY

Catalase present in the suspending medium during irradiation significantly reduced the lethal action of gamma radiation from cobalt-60 for anaerobic bacterial spores. This finding supports the theory that the lethal action of ionizing radiations is at least partially due to secondary effects of the irradiation. Furthermore, such protection of anaerobic bacterial spores is important when sterilization of foods containing catalase is considered since it will likely increase the dosage required.

Two explanations are offered for the lethal action of ionizing radiations on living cells. The first postulates a direct action of the radiations on genetic material; the other assumes that an initial change takes place in the medium or at some nongenetic locus and that this change brings about lethal effects in the cell. The work of Hollander et al. (1951)[7] and Burnett et al. (1951)[8] support the latter theory. Similarly, the work presented in this paper further supports the indirect action theory by describing a protective effect of catalase for anaerobic bacterial spores when catalase is present in the suspending medium during irradiation. Obviously, such protection of anaerobic bacterial spores will probably increase the amount of ionizing radiations required to sterilize foods containing catalase.

MATERIALS AND METHODS

Spores used in this work were grown, harvested, and suspended in distilled water as described by Kempe et al. (1954).[1]

Immediately prior to use in an experiment, the stock spore suspensions were shaken with glass beads for five minutes to disperse the spore clumps. The desired quantity of spores was next pipetted into a sterile test tube and heated at 85°C for 15 minutes to kill the vegetative cells. The spore suspension was then diluted into the final solutions to be irradiated.

The control for these experiments was sterile phosphate buffer to which only the spores of either Clostridium botulinum 213B or Putrefactive anaerobe No. 3679 were added.

Purified crystalline catalase for this work was obtained from General Biochemicals, Inc., Chagrin Falls, Ohio. For use in an experiment,

phosphate buffer (pH 7.02) was sterilized by autoclaving. Following this, 60 mgm of catalase was added to 14.5 ml of the cooled experimental solution and then 0.5 ml of a spore suspension was added to both the control and the catalase solutions.

Four-ml quantities of these preparations were next aseptically pipetted into sterile 5-ml glass vials which were finally sealed in an oxygen flame. Irradiation was carried out in an ice water bath in the center well of the large cobalt-60 source in the Fission Products Laboratory at The University of Michigan.

After completion of the irradiation, a sample from the irradiated or control (0-hours radiation) vial was withdrawn, diluted to the proper spore concentration, and counted using techniques previously described by Reed et al. (1951).[9]

RESULTS AND DISCUSSION

The survival of spores of Clostridium botulinum 213B after varying exposures to Co-60 gamma radiation either in a phosphate buffer solution or in a phosphate buffer solution containing catalase is shown in Table XI and in Fig. 8. In the control solution, only 35 spores per ml remained viable after 8 hours radiation from an original population of 15,000,000 per ml. On the other hand, in the solution containing catalase, 59,000 spores per ml were viable after 8 hours irradiation from an original population of 16,900,000 per ml. This represents a 1500-fold increase in survival caused by the addition of catalase.

Similarly, the effect of catalase on the lethality of Co-60 gamma radiation for the spores of Putrefactive anaerobe No. 3679 is shown in Table XI and in Fig. 8. Here only 145 spores per ml were viable after 7 hours irradiation in the control solution as compared to 179,500 spores per ml after a similar dosage of gamma radiation in the solution containing catalase. This represents a 1250-fold increase in the survival ratio.

Since many investigators, including Allen (1954),[10] have reported that hydrogen peroxide is developed during irradiation of water by gamma rays, and since Curran and Evans (1940)[11] reported that the sporicidal action of hydrogen peroxide could be dissipated by catalase, the inference is drawn that catalase protected the anaerobic spores tested in this work by destroying hydrogen peroxide that was produced in situ by the irradiation treatment. This is evidence for the indirect lethal action of radiation on anaerobic bacterial spores. The magnitude of the observed effect suggests that the sporicidal action of gamma radiation may be largely indirect in nature.

In any event, the protection of anaerobic bacterial spores against the lethal action of ionizing radiations by catalase must be considered when the sterilization of raw foods by such radiations is contemplated.

TABLE XI

THE EFFECT OF CATALASE ON THE LETHALITY OF GAMMA RADIATION FROM COBALT-60 FOR ANAEROBIC BACTERIAL SPORES

	No. of Hours Radiation	Rep*	No. of Organisms Surviving per ml	Log % Survivors
a) *C. botulinum* 213B				
Control	0	0	15,000,000	2.000
	3	465,000	3,500,000	1.368
	5	775,000	136,000	-0.043
	6	930,000	3,330	-1.654
	7	1,085,000	380	-2.596
	8	1,240,000	35	-3.632
Catalase	0	0	16,900,000	2.000
	3	465,000	6,800,000	1.605
	5	775,000	1,690,000	1.000
	6	930,000	710,000	0.623
	7	1,085,000	233,000	0.139
	8	1,240,000	59,000	-0.456
b) PA 3679				
Control	0	0	10,700,000	2.000
	5	775,000	61,500	-0.241
	6	930,000	8,100	-1.121
	7	1,085,000	145	-2.868
Catalase	0	0	11,600,000	2.000
	5	775,000	1,460,000	1.100
	6	930,000	410,000	0.548
	7	1,085,000	179,500	0.1895

*One rep unit is a dose of ionizing radiation capable of producing energy absorption of 93 ergs per gram of tissue.

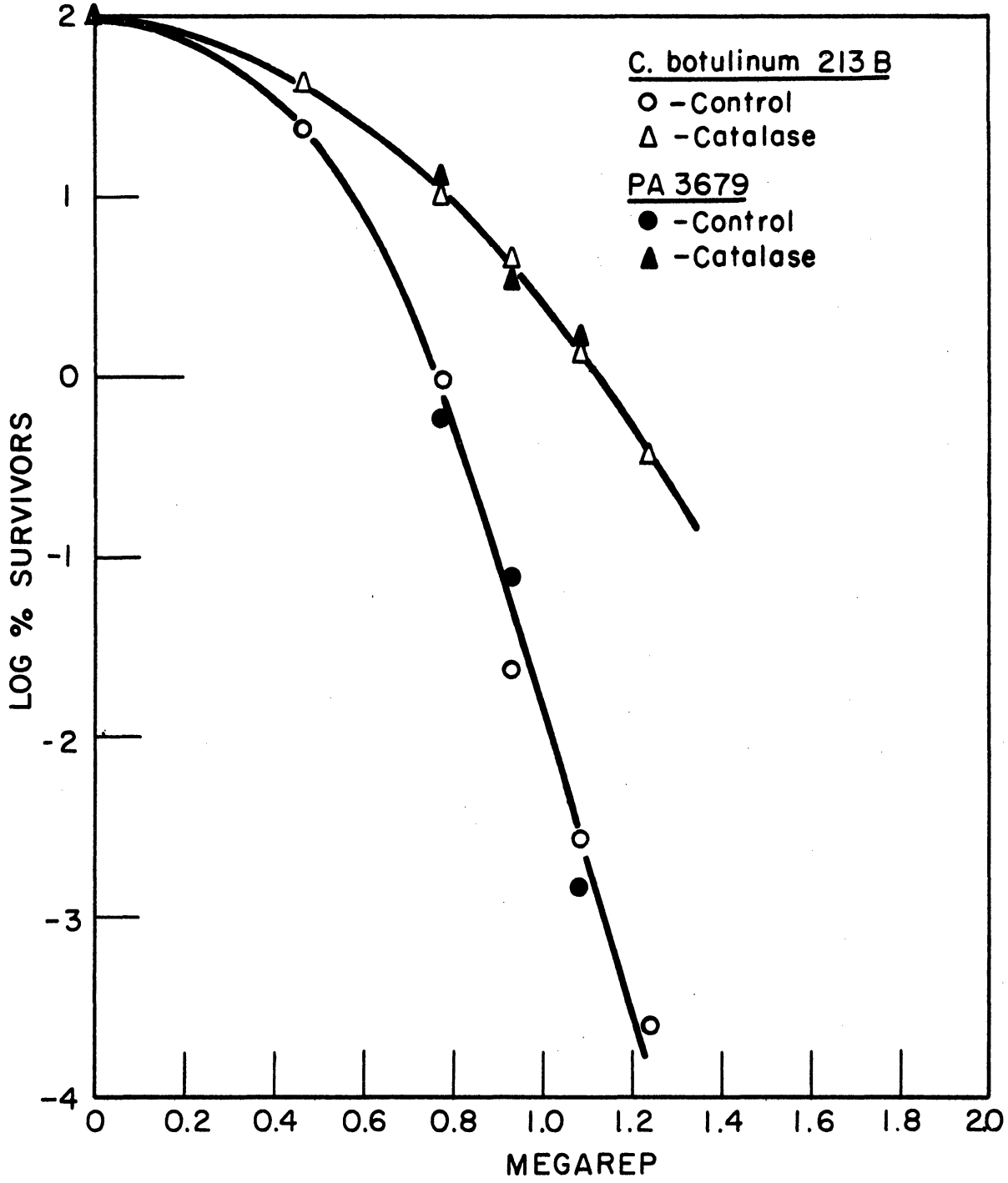

Fig. 8. Effect of catalase on the lethality of gamma radiation from cobalt-60 for anaerobic bacterial spores.

PHASE IV

EFFECT OF PREIRRADIATION OF INOCULATED PACKS OF CANNED PEAS ON THE F_o SUBSEQUENTLY REQUIRED FOR STERILIZATION

All the work under this contract on the combined irradiation—heat processing of canned foods has been based on ground beef. Canned vegetables also might be improved if reduced heat processing is required following irradiation. For convenience in testing, it is desirable to use gas formation as a criterion of growth of anaerobic bacteria. Therefore, peas were selected for study from among the several vegetables that are commercially preserved by canning. Frozen peas were used because fresh peas are only seasonably available.

Although this is a final report, the results from these studies cannot be available for some time—perhaps three months or more—because anaerobic bacterial spores appear to germinate slowly in the canned peas. Therefore, only the method presently in use will be described here. When incubation results are available they will be reported in progress reports of the next project.

EXPERIMENTAL PROCEDURE

Frozen peas are purchased from The University of Michigan Food Service. These are thawed by being placed in a refrigerator over night. They are then dumped into a stock pot, covered with a 1.8% salt, 2.2% sucrose brine, and heated to 200°F. The preliminary heating serves to drive out gases and to pasteurize the peas. Following this, the peas and brine are packed into No. 1 picnic tin cans, and more brine is added if necessary. Some of the cans contain Ecklund thermocouples. Lids are then set on the cans and the cans are placed in an autoclave where they are kept in flowing steam at atmospheric pressure for a few minutes while individual cans are removed, inoculated, and closed. As soon as a can is closed, it is placed in cold, running tap water where it remains for about 20 minutes. Then the experimental cans are either irradiated, or placed in a refrigerator at 35°F. The controls are placed in an 85°F incubator.

Irradiation is carried out in the center well of the large cobalt-60 gamma radiation source here at The University of Michigan. The cans are cooled with dry ice during this irradiation.

Following irradiation, the cans are heat processed and incubated in the same manner as has been previously described for ground beef (see Phase I).

At present, an inoculum of 300 PA 3679 spores per can is being used. With this inoculum, ground beef control cans usually develops hard swells overnight when incubated at 85°F; the canned peas, under these contions require two or three days. It can be expected that the experimental cans will take proportionately longer to develop sufficient gas to indicate positive anaerobic bacterial growth.

As previously stated, no quotable results are available as this is written (middle of July, 1957), but the results will be reported in about three months or more depending upon the incubation time required.

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