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MEDICAL SCHOOL
Department of Bacteriology

Final Report

DETERMINATION OF RADIATION STERILIZATION DOSE FOR CANNED MEAT

1 August 1957 to 30 November 1959

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ABSTRACT

Canned ground beef inoculated with 5,000,000 C. botulinum spores per gram was sterilized with between 3.80 and 3.85 megarad of gamma radiation from cobalt-60. Higher spore concentrations would undoubtedly require higher sterilization dosages.

Botulinus toxin was regularly demonstrated in canned ground beef originally inoculated with 2,670,000 or more C. botulinum 62A spores per gram; these cans were selected from many that remained unswollen during incubated storage for 6 months to 5 years after irradiation sterilization.

Small effects, of both variable temperature at a constant irradiation level and variable irradiation intensity at constant temperature, were shown upon the lethality of gamma radiation for C. botulinum spores.

Combined irradiation-heat processing was shown to be capable of significantly reducing the quantity of each of these forms of energy that are required to sterilize canned green peas. This combined process could offer advantages over sterilization by either process alone. Also, the results with green peas indicate that combined irradiation-heat processing can be applied to vegetables as well as to canned meat. The latter had been previously demonstrated.

OBJECTIVE

The general objective of this study was the development of an improved food-sterilizing process. Determination of radiation dosages required to sterilize canned foods containing known numbers of C. botulinum spores is considered to be necessary before an adequate processing dose can be established. The determination of these dosages for C. botulinum spore concentrations up to 4,000,000 per gram was the principal specific objective of this research.

CONTRACT RESEARCH PROJECT REPORT
QUARTERMASTER FOOD AND CONTAINER INSTITUTE
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HQ, QM RESEARCH AND DEVELOPMENT COMMAND
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Title of Contract: Determination of Radiation Sterilization Dose for Canned Meat

SUMMARY

As set forth in the contractual agreements, studies have been carried out during the past two years to investigate the following:

(1a) To determine the "true" radiation sterilization dose for canned ground meat in the cooked and raw condition using C. botulinum inocula of approximately 4,000,000 spores per gram.

(1b) To determine combined irradiation-heat sterilizing processes for canned, cured meat products. The results obtained are presented in Part II of this report.

It has been found that a dose of 3.8 megarad of gamma radiation sterilized precooked or raw ground beef inoculated with 5,000,000 C. botulinum 213B spores per gram of meat. Cooked ground beef inoculated with 5,000,000 C. botulinum 62A spores per gram of meat was sterilized with 3.85 megarad.

Graphical plots of the data indicate that the linear relationship between radiation sterilization dosages and the logarithm of the spore inocula is valid up to a C. botulinum spore concentration of 5,000,000 per gram. Higher spore concentrations, which are conceivable, would probably require a higher sterilization dosage. This should be investigated.

With canned pork luncheon meat, tentative findings indicate that pres-

ervative chemicals in the product potentiate the lethal action of gamma radiations for anaerobic bacterial spores. Both combined irradiation-heat processing and irradiation alone appear to produce sterility at much lower levels than those found in raw or cooked meat. The long incubation periods involved in these studies make them tedious. Nevertheless, the possibility of lower sterilization dosages seems to make further study desirable both from a theoretical and a practical viewpoint.

(2) To learn whether unswollen cans of meat or vegetable products, inoculated with C. botulinum spores during previous studies and which have been kept in incubated storage, retain any viable spores or active botulinus toxin. Results of the studies conducted for this purpose are discussed in Part III of this report.

Botulinus toxin was regularly demonstrated in canned ground beef originally inoculated 2,670,000 or more C. botulinum 62A spores per gram. This meat was taken from stored, unswollen cans that had been radiation-sterilized and stored in this laboratory for periods of 6 months to 5 years. The toxin found was apparently in the spores when inoculated.

No toxin was found in unswollen cans of meat from previous "sterilization" experiments where C. botulinum 213B or less than 2,670,000 C. botulinum 62A spores were used per gram of meat. Likewise, no clear-cut evidence of either spore germination or aborted vegetative growths were found in the meat in these cans.

The significance of finding toxin in cans of ground meat treated with up to 4.9 megarad of gamma radiation should be further studied.

(3) To determine the effects of variation in temperature at a constant irradiation level and of variation in the intensity of irradiation at constant temperature upon the lethality of gamma radiation for anaerobic bacterial spores. The results of these studies are given in Part IV.

A small but significant effect of temperature during irradiation on the lethality of gamma rays for Clostridium botulinum spores was shown.

Likewise, a small, unexpected effect of radiation intensity also appears to exist. Gamma rays were found to be about 10% less effective at a field intensity of 0.500 than at 0.002 megarad per hour. This finding needs further study.

In addition to the specific contract requirements, some work initiated under a previous contract was completed. Studies of the effect of pre-irradiation of canned green peas on the F_0 subsequently required for sterilization were completed. The results are given in Part I.

Combined irradiation-heat processing is synergistic for killing anaerobic bacterial spores inoculated into canned green peas. Following 1.2 megarad of gamma irradiation, an F_0 of 0.5 sterilized green peas inoculated with either 5,000,000 Clostridium botulinum 213B or 300 PA 3679 spores per can. This processing schedule must be considered a minimum since it is based on a limited number of cans. Because sterilization by either irradiation or heat alone can damage the organoleptic properties of canned foods, the lowered schedules possible with combined processing may prove to be of value for preserving vegetables as well as meats.

PART I

EFFECT OF PRE-IRRADIATION OF CANNED GREEN PEAS ON THE F_o SUBSEQUENTLY REQUIRED FOR STERILIZATION

SUMMARY

Combined irradiation-heat processing is shown to be synergistic for killing anaerobic bacterial spores inoculated into canned green peas. Following 1.2 megarad of gamma irradiation, an F_o of 0.5 sterilized green peas inoculated with either 5,000,000 Clostridium botulinum 213B or 300 PA 3679 spores per can. This processing schedule must be considered a minimum since it is based on a limited number of cans. Because sterilization by either irradiation or heat alone can damage the organoleptic properties of canned foods, the lowered schedules possible with combined processing may prove to be of value for preserving vegetables as well as meats.

INTRODUCTION

While assembling equipment, etc., to study the radiation sterilization dose for canned meat, it was decided to complete a previously initiated effort to determine the combined irradiation-heat processing schedules for canned green peas.

Radiation and heat have previously been shown to be synergistic in their lethal action on spores of bacteria that cause food spoilage. This study was directed towards utilizing this synergism in food preservation by determining the various combinations of heat and irradiation required to kill spores of Clostridium botulinum and PA 3679 that were purposely inoculated into canned ground peas.

Green peas are particularly suitable for study because they are continually available on the open market in the frozen condition; also they are an excellent recovery medium for Clostridium botulinum and PA 3679 spores, as well as being a staple item of the human diet. Thus they afford a good system with which to develop techniques and still produce usable information.

MATERIALS AND METHODS

Frozen green peas were obtained in 2-1/2-lb boxes from the stock supply of The University of Michigan Food Service. These peas carried the

label of the Frost Queen Packing Company of Tacoma, Washington. The packages indicated that the peas contained a "slight amount" of added salt.

In preparation for a run, 15 lb of the frozen peas were placed in a stock pot and then were covered with a brine containing 1.8% sodium chloride and 2.2% sucrose. This brine has previously been described for peas by Reed et al.[1] The stock pot was then placed in a boiling water bath for about 1-1/2 hr during which time the frozen peas melted and were brought to a temperature of 205°F.

No. 1 Picnic tin cans were filled with these peas; care was taken to cover the peas with brine. Covers were then placed loosely on the cans and these were set in an autoclave which was kept filled with flowing steam. After the cans had been exhausted for a few minutes in the flowing steam, individual cans were removed from the autoclave, inoculated with one ml of a spore suspension, sealed in a commercial-type closing machine, dumped into cold, running water for 20 minutes, and then refrigerated until they were either irradiated, heat-processed, or incubated as required.

Irradiation.—The canned peas were irradiated in the center-well of the large cobalt-60 gamma radiation source in the Fission Products Laboratory at The University of Michigan.[2] The quantity of irradiation delivered at the center of the cans were measured by ferrous-ferric sulfate dosimetry as previously described.[3] At the time of this investigation, a dosage of 1* megarad of gamma radiation required approximately an 8-hr exposure. When the temperature of the cave was above 40°F, the cans were refrigerated with dry ice to keep the peas below this temperature during irradiation. Following irradiation the cans were placed in a refrigerator at 35°F from which they were removed within 2 days for the heat-processing.

Heat Processing.—Six cans of meat, two of which contained thermocouples, were placed in an autoclave where they were heat-processed to the desired F_0 values as previously described.[4] The F_0 value is defined as the number of minutes required to sterilize the can of peas at 250°F when the Z value equals 18. These values were calculated from time-temperature curves obtained from the cans containing thermocouples. For this purpose Schultz's graphical modification of Ball's General Method[5] was used, and a Z value of 18 was assumed. Following heat-processing, the cans were incubated at 85°F.

Spores.—The spores of anaerobic bacteria used in these studies were prepared and used as previously described.[3]

*One rad is a dose of ionizing radiation capable of producing energy absorption of 100 ergs per g of tissue.

Controls.—Eight control cans were used. Four of these were not inoculated and four were selected at random from the experimental cans. All eight were then incubated at 85°F. Generally the noninoculated cans swelled within three weeks while gas usually developed within one week in the inoculated cans. This indicated that conditions suitable for microbiological growth were present. However, this did not conclusively demonstrate viability of the inoculum in the peas, since the noninoculated peas contained bacteria.

When Clostridium botulinum 213B was used for the inoculum, mouse inoculation tests established the presence or absence of B type toxin in the peas.[3] Occasionally this type of toxin was also recovered from noninoculated control cans which indicated that the peas contained C. botulinum type B spores when originally frozen. For this reason, the F_0 required to sterilize noninoculated canned peas was established by control runs.

RESULTS

Data for a typical run are shown in Table I-1. Two series of runs were conducted. In the first series, summarized in Table I-2 and Fig. I-1, 5,000,000 C. botulinum 213B spores were used per No. 1 can of peas. In the second series, summarized in Table I-2 and Fig. I-2, 300 PA 3679 spores were similarly used. Itemized data are given in Appendix A. These data show that gamma radiation and heat are synergistic when used together for processing canned peas. Less of either form of energy was needed for sterilization when combined with the other than when used alone. Following 1.2 megarad of gamma radiation, an F_0 of 0.5 sterilized the peas whether they were inoculated with 5,000,000 C. botulinum 213B or 300 PA 3679 spores. Below 1 megarad pre-irradiation, more heat-processing was required to sterilize cans of peas inoculated with 300 PA 3679 spores than was necessary when 5,000,000 C. botulinum 213B spores were used; above 1 megarad pre-irradiation, the reverse was true.

DISCUSSION

The synergistic lethal action of gamma radiation is probably a general phenomenon since it is shown to be essentially as pronounced for spores suspended in canned green peas as it has previously been reported to be for such spores suspended in canned beef and in phosphate buffer.[4,6] It would therefore appear reasonable from a microbiological viewpoint that this synergistic lethal property could be expected to be applied to the sterilization of most canned foods. It also appears that the synergistic action results from sensitization of the spores to heat as a result of irradiation. This effect varies in degree according to the medium in which the spores are

suspended, so wherever utilization of the effect is considered, the actual application should be studied.

It must be pointed out that the combined irradiation—heat-processing treatments reported here are minimum values because they are based on a limited number of runs and upon four cans at each level as shown in Table I-1.

Relevant to the possible improvement in organoleptic values to be derived from combined irradiation—heat-processing, Gillies[7] has recently reported on studies with canned peas. He found that thermally processed peas were superior to those processed either by gamma radiation alone or by combined irradiation—heat-processing. However, he also stated that combination-processed peas were significantly better, in most cases, than those processed with radiation alone.

TABLE I-1

F_o VALUES REQUIRED TO STERILIZE CANNED GREEN PEAS PACKED IN NO. 1 PICNIC TIN CANS, INOCULATED WITH PA 3679 SPORES, AND IRRADIATED WITH GAMMA RAYS FROM COBALT-60 BEFORE HEAT-PROCESSING

Run No. CP 6	
Can Size	No. 1 Picnic (211 x 400)
Product	Green Peas
Inoculum	300 PA 3679 spores per can
Irradiation	0.279 megarad
Processing temperature	230°F
Incubation temperature	85°F

Fo	Can No.	Days-to-Gas Formation
0.77	13	4
0.77	14	4
0.77	15	4
0.77	16	4
1.47	17	5
1.47	18	4
1.47	19	4
1.47	20	5
2.19	21	-
2.19	22	5
2.19	23	-
2.19	24	-
3.16	9	-
3.16	10	-
3.16	11	-
3.16	12	-
Controls:	1	3
Not inoculated	2	-
	3	23
	4	6
Controls:		
Inoculated	5	3
	6	3
	7	6
	8	7

Conclusions: Following 0.279 megarad of gamma radiation from cobalt-60, canned peas were sterilized by an F_o between 2.2 and 3.2.

TABLE I-2

COMBINED IRRADIATION—HEAT-PROCESSING TREATMENTS REQUIRED TO STERILIZE CANNED GREEN PEAS PACKED IN NO. 1 PICNIC TIN CANS AND INOCULATED WITH ANAEROBIC BACTERIAL SPORES

Run No.	Pre-irradiation, megarad	F_o range, min
a. Series 1 - 5,000,000 C. botulinum 213B spores per can		
PB 6	2.33-2.79	None
PB10	>2.79	None
PB19	2.79-3.29	None
PB11	None	<1.54
PB 4	None	1.39-1.82
PB 1	None	>1.44
PB 3	0.465	1.03-1.53
PB14	0.465	0.60-0.75
PB15	0.650	0.80-0.92
PB 5	0.930	0.36-0.50
PB 8	1.40	0.33-0.48
PB18	1.63	<0.30
PB17	1.96	<0.06
b. Series 2 - 300 PA 3679 spores per can		
CP 2	1.77-2.05	None
CP 1	None	4.1-4.9
CP 6	0.279	2.2-3.2
CP 3	0.465	1.39-2.25
CP 5	0.697	0.33-0.69
CP 4	0.930	0.25-0.49
Noninoculated controls		
PB 7	None	>0.06
PB 9	None	>0.054
PB12	None	0.35-0.52
PB13	None	>0.20
PB15	None	0.10-1.10
PB 7	>0.465	None
PB15	>0.650	None

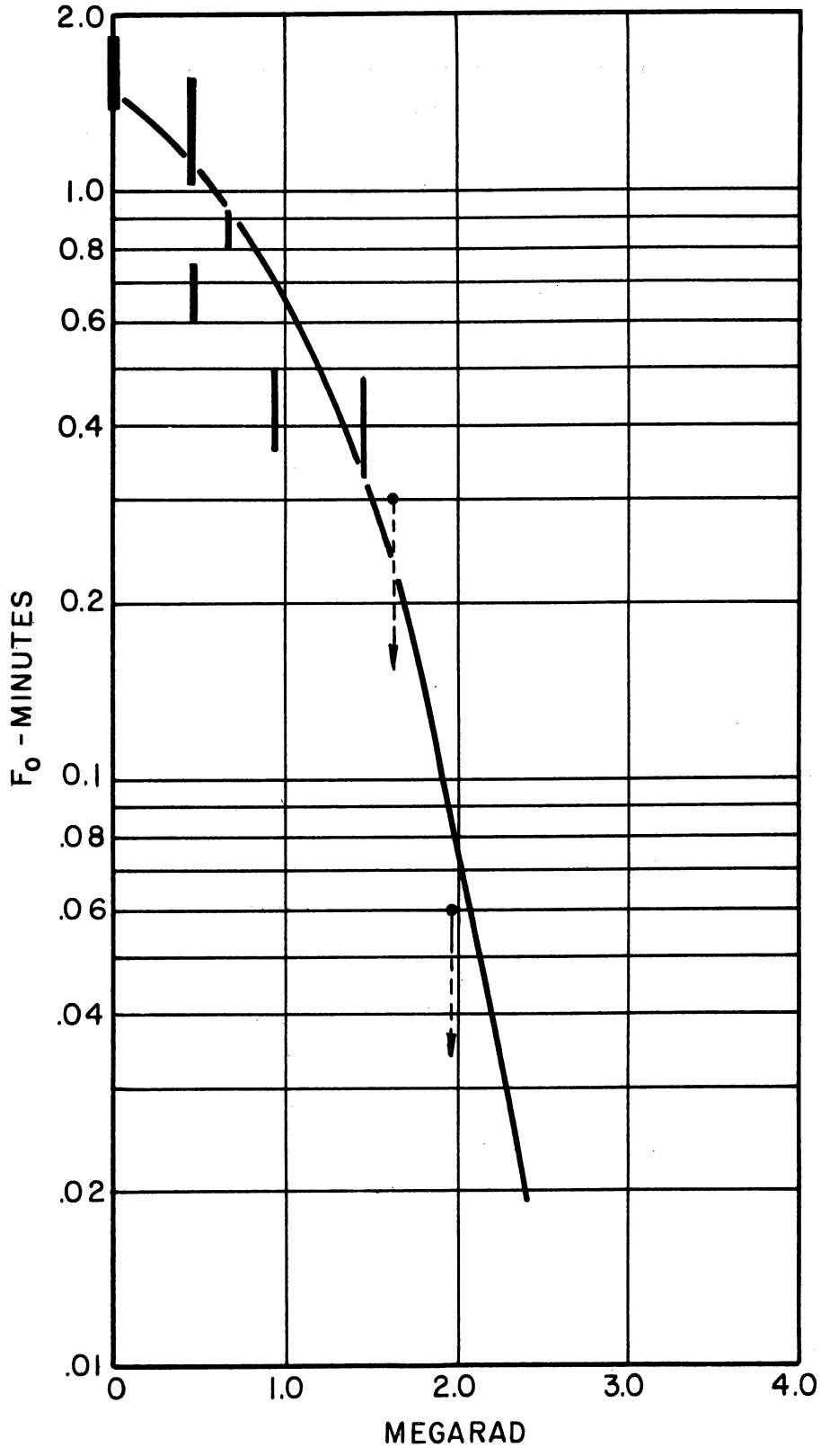

Fig. I-1. F_o required to sterilize green peas packed in No. 1 picnic tin cans, inoculated with 5,000,000 C. botulinum 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat-processing at 230°F and incubating at 85°F.

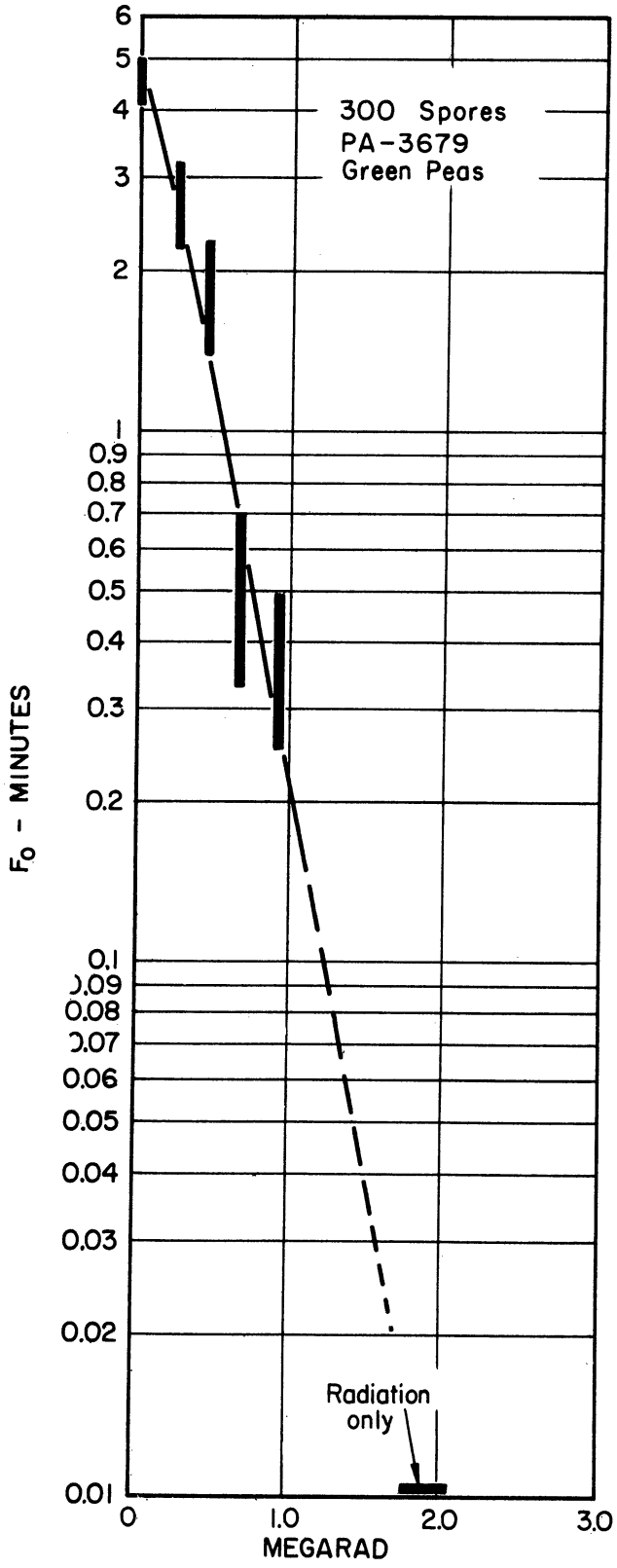

Fig. I-2. F_o required to sterilize green peas packed in No. 1 picnic tin cans, inoculated with 300 PA 3679 spores per can, and irradiated with gamma rays from cobalt-60 before heat-processing.

PART II

DETERMINATION OF RADIATION STERILIZATION DOSE FOR CANNED MEAT

1. Ground Beef, Pre-Cooked and Raw

SUMMARY

A dose of 3.8 megarad of gamma radiation sterilized pre-cooked or raw ground beef inoculated with 5,000,000 C. botulinum 213B spores per gram of meat. When 5,000,000 C. botulinum 62A spores were used per gram of cooked ground beef, the sterilizing dose was found to be 3.85 megarad.

These data extend our information regarding sterilizing dosages from that previously reported for an upper level of 40,000 spores per gram to a concentration of 5,000,000 spores per gram. Furthermore, the previously reported linear relation between sterilizing dosages and the logarithm of the spore concentration was shown to continue to the 5,000,000 spores per gram level. These data do not indicate that "the" radiation sterilization dose has been determined. C. botulinum spore concentrations higher than 5,000,000 per gram are conceivable in meat. Furthermore, statistical considerations suggest that a much larger number of spores should be considered for this purpose; this has been done in determining heat-sterilization criteria.

INTRODUCTION

Previously reported data[3] showing the amount of gamma radiation required to sterilize ground beef packed in tin cans have been extended to include C. botulinum spore concentrations of one million per gram. This part of the report presents data for both cooked and raw ground beef packed in mushroom-style tins and inoculated with C. botulinum 213B spores, as well as some data for C. botulinum 62A spores that were similarly used.

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 213B spore suspensions were prepared according to procedures described by Reed et al.[1] except that Difco bacto-castone was substituted for casein digest in the medium specified by these workers.

The C. botulinum 62A spores were grown in the liver broth medium described by Reed et al.[1] 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.[1] For this purpose 0.1% soluble starch was incorporated into the pork agar medium to aid germination of the spores.[8]

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 mushroom-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 irradi-

ated, 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 itemized in Table II-1,* summarized in Table II-2, and plotted in Fig. II-1. As would be expected from previously reported results,[1] the sterilizing dosage varies directly with the logarithm of the number of C. botulinum 213B 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 213B spores per gram. Since C. botulinum 213B spores were previously[3] found to be slightly less resistant to gamma radiation than C. botulinum 62A 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. II-2 and Tables II-2 and II-4,* 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 213B 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. II-1 for cooked beef. This, in our opinion, is caused by problems associated with the native bacterial flora of raw ground meat.

The radiation sterilization dosage for both cooked and raw ground beef, inoculated with C. botulinum 62A spores, is itemized in Table II-3* and summarized in Table II-2. These data indicate that cooked ground beef, inoculated with approximately 5,000,000 C. botulinum 62A spores per gram, was sterilized by 3.85 megarad of gamma radiation. Similar results were obtained with raw ground beef under these conditions.

It should not be inferred from these results that "the" radiation sterilization dose has been determined. Actually, higher inocula could be used in individual cans and the inocula of individual cans could be interpreted collectively in a much grander type of experiment. Such an experiment would probably indicate a higher irradiation level as the upper

*See Appendix B.

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

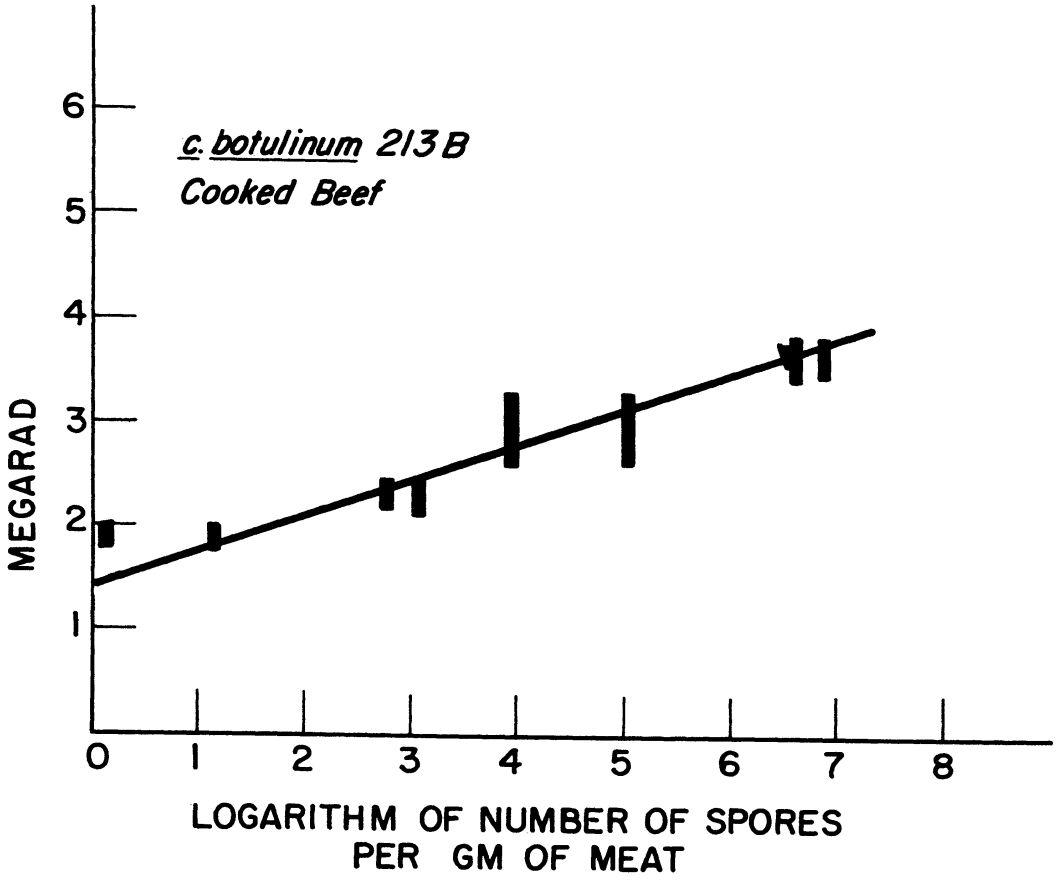

Fig. II-1. The dosage of gamma radiation from cobalt-60 required to sterilize cooked ground beef containing spores of C. botulinum 213B.

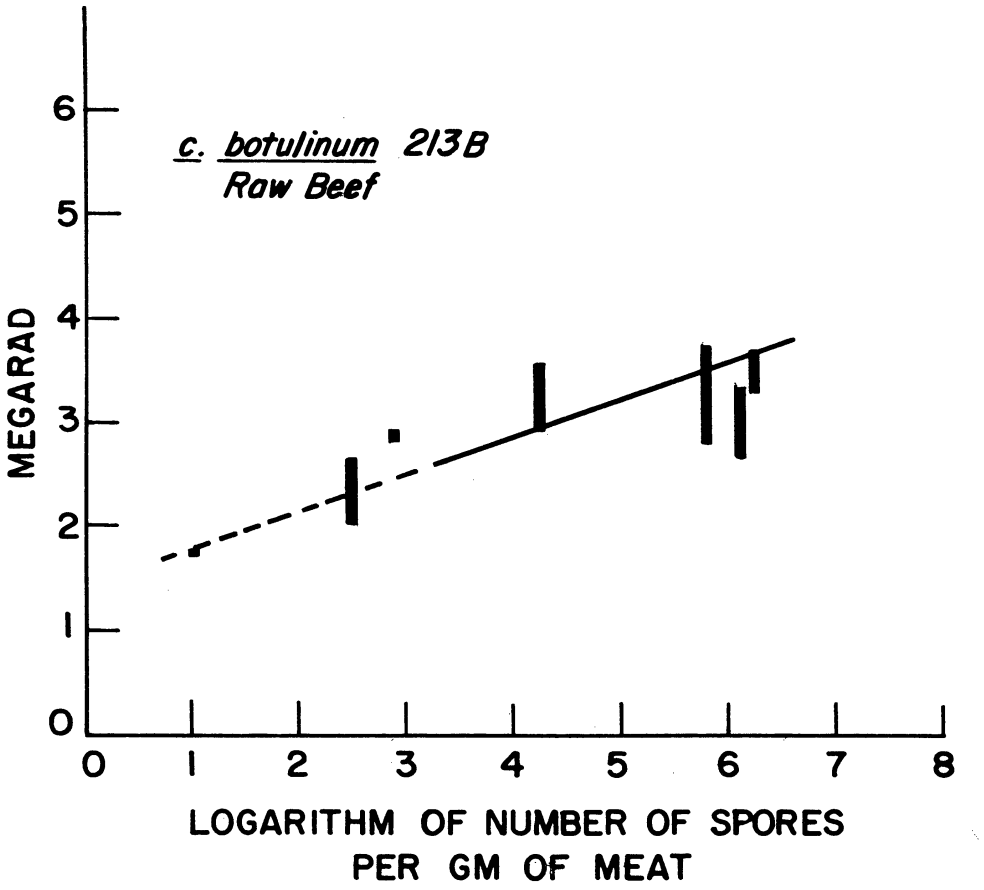

Fig. II-2. The dosage of gamma radiation from cobalt-60 required to sterilize raw ground beef containing spores of C. botulinum 213B.

TABLE II-2

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 g 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
A-5	3,300,000	3.10-3.35
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

limit or sterilization dose. Also, there are competent research workers who question the value of inoculated packs for determinations of the sterilization dose. These workers seem to feel that spores produced in the meat, as they would be by growth in the food, might have a different resistance to radiation. Perhaps this also should be tested. However, it should be noted that commercial heat-sterilization levels for canned foods are based on inoculated pack data and these have been quite successful in determining heat-sterilization processes for routine industrial use.

2. Determination of Irradiation and Combined Irradiation—Heat-Processing Treatments Required to Sterilize Canned Pork Luncheon Meat

SUMMARY

Limited data indicate that the preservative chemicals in pork luncheon meat potentiate the lethal action of gamma radiation for anaerobic bacterial spores. This tentative finding should be tested further since it appears possible that commercial sterility may be attained in pork luncheon meat at dosages much below those required for raw meat. Likewise the amount of combined irradiation—heat-processing, or indeed of heat-processing alone, also appears to be significantly lower for attainment of sterility in pork luncheon meat than in unpreserved meat. This, of course, is really not unexpected but may be, nonetheless, significant.

The refrigerated storage lives of pork luncheon meat, bacon, etc., are considerably greater than those of the fresh meats from which they are made. The preserving chemicals, used in their preparation, appear to be reasonably efficient in this regard even when pork luncheon meat is incubated at 85°F. This study was undertaken to learn whether the presence of preserving chemicals in pork luncheon meat, together with small dosages of gamma radiation, would permit reliable room-temperature storage of the meat.

MATERIALS AND METHODS

Pork luncheon meat was obtained from Swift and Company through the courtesy of Dr. W. M. Urbain. The product was furnished from one batch and was reported to have the following composition:

	Percent by Weight
Pork	90.9
Salt	3.6
Sucrose	2.7
Sodium nitrate	0.014
Sodium nitrite	0.007
Spice	0.028
Water	2.7

The meat was packed in 6-lb square tins marked as a perishable product and labeled "Savor-tite Pure Pork Luncheon Meats." The unopened cans were kept at 40°F until used.

Our re-preparation of the meat for canning varied because difficulty was encountered in getting the PA 3679 spores to grow and develop gas during incubation at 85°F. In the beginning this difficulty was observed even with the inoculated controls. Following a suggestion of Dr. Urbain of Swift and Company, we took increasingly rigorous precautions to remove dissolved oxygen. This included evacuation in runs after No. 3 and nitrogen packing after No. 6. A detailed description of the meat preparation follows:

Run LPA 1.—The luncheon meat was removed from the refrigerator, taken from the cans and ground in a commercial-type grinder. This ground meat was then packed in No. 1 picnic tin cans, which were covered loosely with covers and autoclaved at 121°C for 1 hr. Individual cans were removed from the autoclave, inoculated at the geometrical center of the meat with 1 ml of a spore suspension containing 300 PA 3679 spores per ml, sealed in a commercial-type closing machine, and plunged into cold running water. Here they cooled to about 65°F in a few minutes and were then further cooled to 33°F in a refrigerator. Following this the cans were either heat-processed or incubated at 85°F as indicated.

Run LPA 2.—This was the same as LPA 1 except that 10,000 PA 3679 spores were used per ml of spore suspension.

Run LPA 4.—The meat was ground as for previous runs but was packed directly into cans. These were inoculated with 1 ml of spore suspension containing 10,000 PA 3679 spores per ml, and then sealed in a commercial-type, vacuum closing machine at a vacuum of 29 in. of Hg. The meat remained cold throughout this treatment. Following this, the cans were processed, and refrigerated at 33°F or immediately incubated at 85°F, as indicated.

Run LPA 5.—Cold ground pork luncheon meat was spread into shallow enamelled-ware pans to about a 2-in. depth and then placed in an evacuation chamber. Here the pressure was reduced to 25 in. of Hg and then nitrogen was introduced to restore atmospheric pressure. The nitrogen was allowed to remain in contact with the meat for five minutes. This was repeated three times. Next the ground beef was packed into No. 1 picnic tin cans and three more cycles of the evacuation and nitrogen replacements process were applied. Following this, 10,000 PA 3679 spores were injected into the geometrical center of the meat in each can. These spores were suspended in 10 ml of distilled water. Covers were then positioned and the cans were sealed at 29 in. of Hg vacuum using a commercial-type vacuum closing machine. For processing, the canned pork luncheon meat was irra-

diated in the centerwell of the large cobalt-60 source at The University of Michigan where the dosage at the center of the cans was 125,000 rep per hr at this time. Irradiation was followed by heat processing or immediate incubation as required.

Run LPA 6.—This was the same as LPA 5.

Run LPA 7.—This was also the same as LPA 5 with one exception; after each evacuation, sufficient nitrogen gas was released into the chamber to develop a pressure of 15 psig. The meat remained under this nitrogen pressure for 5 min as part of each of the total of six evacuation cycles.

Run LPA 8 and 9.—These were the same as LPA-7.

Run LB 1.—Same as LPA 7 except that an inoculum of 1,000,000 C. botulinum 213B spores was used per can of meat.

RESULTS AND DISCUSSION

Putrefactive anaerobic bacterial spores do not develop cultures easily in pork luncheon meat even when incubated at 85°F. This is to be expected since such meat contains salt, nitrites, etc., that are added as preservatives. However, by removing dissolved oxygen, bacterial growth was regularly obtained in control cans of Run LPA 5 et seq.

The results must be considered preliminary and, indeed, fragmentary. However, the data, based on approximately 18 months' incubation, suggest sterilization limits for pork luncheon meat, inoculated with 10,000 PA 3679 spores per No. 1 picnic can as summarized in Table II-6. Detailed data are given in Appendix B, Table II-5.

TABLE II-6

COMBINED IRRADIATION-HEAT-PROCESSING TREATMENTS REQUIRED TO STERILIZE PORK LUNCHEON MEAT

Run No.	Pre-irradiation, megarad	F_o range
a) Inoculated with 10,000 PA 3679 spores per can		
LPA 2	None	1.1-3.2
LPA 5	None	<0.86
LPA 7	1.68	<0.17
LPA 8	<0.93	None
LPA 9	None	1.1-3.5
b) Inoculated with 1,530,000 C. botulinum 213B spores per can		
LB 1	>0.744	None

There is some indication from these results that the preservative chemicals lower the amount of irradiation or heat or the severity of combined irradiation—heat-processing that is necessary to produce commercial sterility; it seems desirable to pursue this lead with further study. Certainly something is accomplished in the preservative process for pork luncheon meat that potentiates the lethality of irradiation as a commercial sterilizing agent. However, such a study would require considerable time because long incubations are needed before conclusions can be reached.

PART III

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

SUMMARY

Botulinus toxin was regularly demonstrated in canned ground beef originally inoculated with 2,670,000 or more C. botulinum 62A spores. The amount of radiation used up to 4.9 megarad, the highest level studied, had no effect on the presence of toxin. A separate study of this finding showed that 10^8 spores of this variety were marginally fatal to mice; 10^9 spores were definitely fatal for these animals. The spores were "heat-shocked" before use. Such a heat treatment inactivates "external" toxin.

When C. botulinum 213B spores or less than 2,670,000 C. botulinum 62A spores per gram of meat were used, toxin was not demonstrated in any of the cans tested. Furthermore, no clear-cut evidence of either spore germination or aborted vegetative growths was found.

The significance of the finding of toxin from residual "dead" spores should be investigated further.

INTRODUCTION

The possibility that irradiation-sterilized meat may still contain or develop botulinus toxin after irradiation-processing has been a matter of concern. Two possible sources of such toxin are described as follows:

(a) Botulinus toxin may have formed during growth of a C. botulinum culture in the meat before irradiation-processing. Since the amount of irradiation needed to inactivate botulinus toxin is several times that needed for sterilization, such toxin could remain after processing.

(b) Botulinus spores may have been "killed" by irradiation but still be capable of germination and growth through one or two vegetative cycles. In this case, the question arises whether botulinus toxin would result, and if so, whether enough would be formed to be detectable.

To examine these possibilities, inoculated cans of ground beef that had previously been sterilized by gamma radiation and then incubated in our laboratory were tested. Some of this ground beef was radiation-sterilized as early as 1953. These cans were opened and their contents were examined.

MATERIALS AND METHODS

Canned ground beef for analysis is selected from among those cans of inoculated packs which have been stored the longest; of these, cans remaining unswollen from groups that received approximately the minimum radiation sterilization dose are selected. Our definition of the irradiation sterilization dose, for any particular experiment, is that dose at which none of the cans processed at that or any higher dosage swelled during subsequent incubation. In some instances, cans of meat remain unswollen from groups that received just less than the sterilizing dose. We prefer to use these.

For examination, the cans are first scrubbed with a water-detergent mixture; then they are dried and placed in an enameled pan on a towel soaked in a cresylone solution. The depression in the top of the can is then filled with 95% ethyl alchohol which is ignited and allowed to burn off in order to sterilize the can lid. At the same time, the sides of the can are warmed with the flame from a Bunsen burner in order to neutralize any remaining vacuum. At this point, care is taken not to overheat the can since botulinus toxin is heat-labile. A sterile pad of cotton is next aseptically placed over the can in preparation for finally releasing any vacuum, or slight pressure that may exist. This is accomplished by punching a small hole in the can cover with a sterile ice pick that is pushed through the pad and then through the cover. The can cover is next removed with a sterile can opener.

Four samples of meat are now taken, using a sterile tube and plunger-type sampler. The samples are removed from the interior contents of the can, which could not have been warmed during the opening proceedings. The three samples to be used for subculturing weigh approximately 15 g each; the sample for toxin analysis is slightly less than 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 at least 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 tested 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 1 ml intraperitoneally into each of four 10- to 15-g mice. A tentatively positive finding is indicated by death of the mice within 72 hours. If one mouse dies out of four, the sample is rechecked using a series of three more mice. If further studies are necessary, they are carried out with the specific-type botulinus antitoxin. This involves intraperitoneal injection into mice of portions of the sample which first has been incubated over night in

a refrigerator with the specific-type antitoxin.

The larger, approximately 25-g sample is pushed into a sterile test tube and an approximately equal volume of physiological saline is added. Alternatively, for some of the tests, instead of using 25 g of meat for toxin analysis, all the meat remaining after samples are removed for subculturing is used. This provides about 75 g instead of 25 g of meat for this purpose.

The meat and saline are aseptically mixed and allowed to infuse in a refrigerator for a few hours. The supernatant liquid is then aseptically filtered through a glass-wool pad and 1/2-ml portions of the filtrate are injected intraperitoneally into each of four 10- to 15-g mice. If no mice die within 72 hours, the sample is assumed to be nontoxic. Should one or more mice die in this interval, 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.

The cans of meat tested usually were selected either from the cans remaining unswollen in that group which received the highest dosage in a run and still had some swollen cans in the group, or from the group that received the lowest dosage in which all the cans remained unswollen upon incubation.

RESULTS AND DISCUSSION

Description of Samples for Analysis.—Cans of ground beef were removed from room-temperature storage. These were irradiation-sterilized over the past 6 years and have been incubating 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.[3]

The finding of a few Gram-positive rods 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 data available strongly suggest that, if such cells do develop, they do not liberate enough toxin into the broth to make it toxic for mice.

The data summarized in Tables III-1 and III-2* show that botulinus toxin was regularly present in 75-g portions of meat from unswollen cans

*See Appendix C.

that had been inoculated with more than 2,670,000 C. botulinum 62A spores per can of meat before irradiation, incubation, and storage. When inocula less than 1,000,000 such spores per gram of meat were used and 25-g samples were analyzed, the presence of botulinus toxin was not demonstrated. Some points need emphasis in this connection, viz.: Where toxin was demonstrated in the canned meat,

(1) The spores used for inoculation were all C. botulinum 62A, grown in liver extract medium. These spores were washed at least 12 times with distilled water before storage in distilled water at 4°C. They were taken from different spore crops.

(2) Before inoculation into cans of meat, all spore suspensions were heat-shocked at 85°C for 15 min. This inactivated any toxin that may have "leaked" from the spores during storage.

(3) No classical evidence of growth was found by microscopic examination of the cans, although this does not exclude possible spore germination followed by one or two vegetative cell divisions.

(4) No viable spores were found as was evidenced by lack of growth in the subculture media.

(5) All the cans containing toxin had been inoculated with more than 10^6 C. botulinum 62A spores per gram of meat. This means that more than 10^8 spores were used per can in these instances.

(6) Approximately 75 g of the original 90 g of meat in the can were analyzed.

From previous work, it is known that mice can often be killed with heat-shocked C. botulinum spores. We therefore inoculated mice with varying concentrations of C. botulinum 62A spores that had been heat-shocked for 15 min at 85°C. The results are shown in Table III-3. For this experiment 0.5 ml of a suspension, containing the number of spores indicated per ml, were used. Lethality of the suspensions are recorded as number of mice dying within 72 hours.

The data in Table III-3 show that 10^9 spores, treated in the way that they were used for inocula, killed mice; 10^8 spores were marginally fatal and 10^7 spores were tolerated. These data also suggest that spores receiving the entire processing in the canned meat may have contained more active toxin than did the original spores. If this is indeed the case, it would be interesting to know the reason. For example, did irradiation increase the toxicity of the spores? It should be noted, however, that about 10^8 spores were inoculated into each can—barely enough, by our tests, to kill a mouse by botulinus poisoning.

TABLE III-3

EFFECT OF INTRAPERITONEAL INOCULATION OF C. BOTULINUM 62A SPORES INTO MICE

Number of Spores Inoculated into Each Mouse	Lethality*	Remarks
3.4×10^9	3/3	--
3.4×10^8	1/3	--
3.4×10^7	0/3	--
Controls:		
3.4×10^9	0/3	Autoclaved 30 min
3.4×10^9	4/4	Irradiated, 3.25 megarad

*In other similar experiments, neutralization tests showed that death of mice under these conditions was due to C. botulinum toxin type A.

It was observed that the toxin recovered from the incubated cans was heat-labile. This means that its condition had changed from the time when it was inoculated since the spores were heat-shocked before injection.

It will also be noted in Tables III-1 and III-2** that irradiation with as much as 4.9 megarad did not inactivate the toxin.

These, along with other references and conclusions, strongly suggest the need for further investigation of these findings.

**See Appendix C.

PART IV

EFFECT OF IRRADIATION CONDITIONS ON LETHALITY OF GAMMA RAYS FROM COBALT-60 FOR *C. BOTULINUM* SPORES

SUMMARY

A small but significant effect of temperature during irradiation on the lethality of gamma rays for *Clostridium botulinum* spores was shown. Protection occurred in the temperature range of 40 to 80°C. The magnitude of the effect is about 10%.

Likewise, a small effect of radiation intensity also appears to exist. Gamma rays were found to be about 10% less effective at a field intensity of 0.500 than at 0.002 megarad per hour. This latter finding must still be considered tentative.

The finding of an intensity of irradiation effect was unexpected. It needs verification by further study.

INTRODUCTION

The lethality of ionizing radiation for the spores of *Clostridium botulinum* has previously been reported by us[9] to vary with the temperature during irradiation. This variation was previously reported to be approximately 15% at 0.740 megarep.

MATERIALS AND METHODS

Temperature Effect.—For this purpose, *Clostridium botulinum* 62A spores were grown in liver extract medium at 37°C. They were harvested, washed, and frozen in distilled water and then stored at -40°C until used.

Previous to irradiation, the spores were diluted in M/15 phosphate buffer at pH 7.0 to a concentration of approximately 1,000,000 cells per ml and then pipetted into 5-ml glass vials. These vials were sealed in an oxygen-acetylene flame, and placed in a rack. The rack constitutes part of an apparatus that fits into the center well of the cobalt-60 radiation source in our Fission Products Laboratory. It allows the temperature of the spores to be controlled ±1°C during irradiation over a rather wide temperature range. The spores were then irradiated at various controlled temperatures while suspended in the phosphate buffer. Vials were removed at

desired time intervals and taken to the laboratory for counting. The intensity of the irradiation field was 0.203 megarad per hour during these experiments. The spores were counted by dilution into Prickett tubes containing pork extract agar, followed by incubation at 30°C for 5 days and enumeration of the number of colonies developed.

Irradiation Intensity Effect.—The same spores and counting techniques were used as those described in the previous paragraph. However, screw-capped test tubes (15 x 125 mm) were used to contain the spore suspension rather than the vials.

Irradiation was always carried out in an ice-water bath at 4°C. Also, instead of removing a vial at each interval of irradiation time, a portion of the sample was pipetted from the test tube into a bottle of dilution water. This was then taken to the laboratory for counting.

Two cobalt-60 gamma ray sources were used. One source is located in the Fission Products Laboratory, the other in the Phoenix Laboratory. Both are situated on The University of Michigan campus. The Fission Products Laboratory source was used for intensities between 2000 and 200,000 rad per hour; the Phoenix source was used for the higher intensity of 591,000 rad per hour.

RESULTS AND DISCUSSION

Temperature Effects.—The data are presented in Tables IV-1 and IV-2, and Fig. IV-1, and in the figures in Appendix D. They corroborate our previous findings.[9] A small but significant effect of the temperature during irradiation was shown. Between -72 and 4°C, there is little if any effect of temperature; however, between 40 and 80°C, the spores are protected, gamma radiation being about 10% less effective in this range. At 85°C the combined irradiation-heat effect develops and results in a very pronounced increase in the rate of spore killing. This study is not complete; there were not enough funds to finish it.

Effect of Irradiation Intensity.—The data from this study are summarized in Table IV-2 and Fig. IV-2. The results suggest that a small effect of intensity of irradiation on the lethality of gamma rays from Clostridium botulinum 62A spores may exist. As the irradiation intensity was raised from 2000 to 591,000 rad per hour, the efficiency of the irradiation for killing these spores appears to decrease about 10%. But the reality of this effect may not correspond to its appearance. More data are needed to establish its magnitude precisely, and in any event, the effect does not appear to be large. Due to termination of the contract, no further work was carried out on this phase of the study.

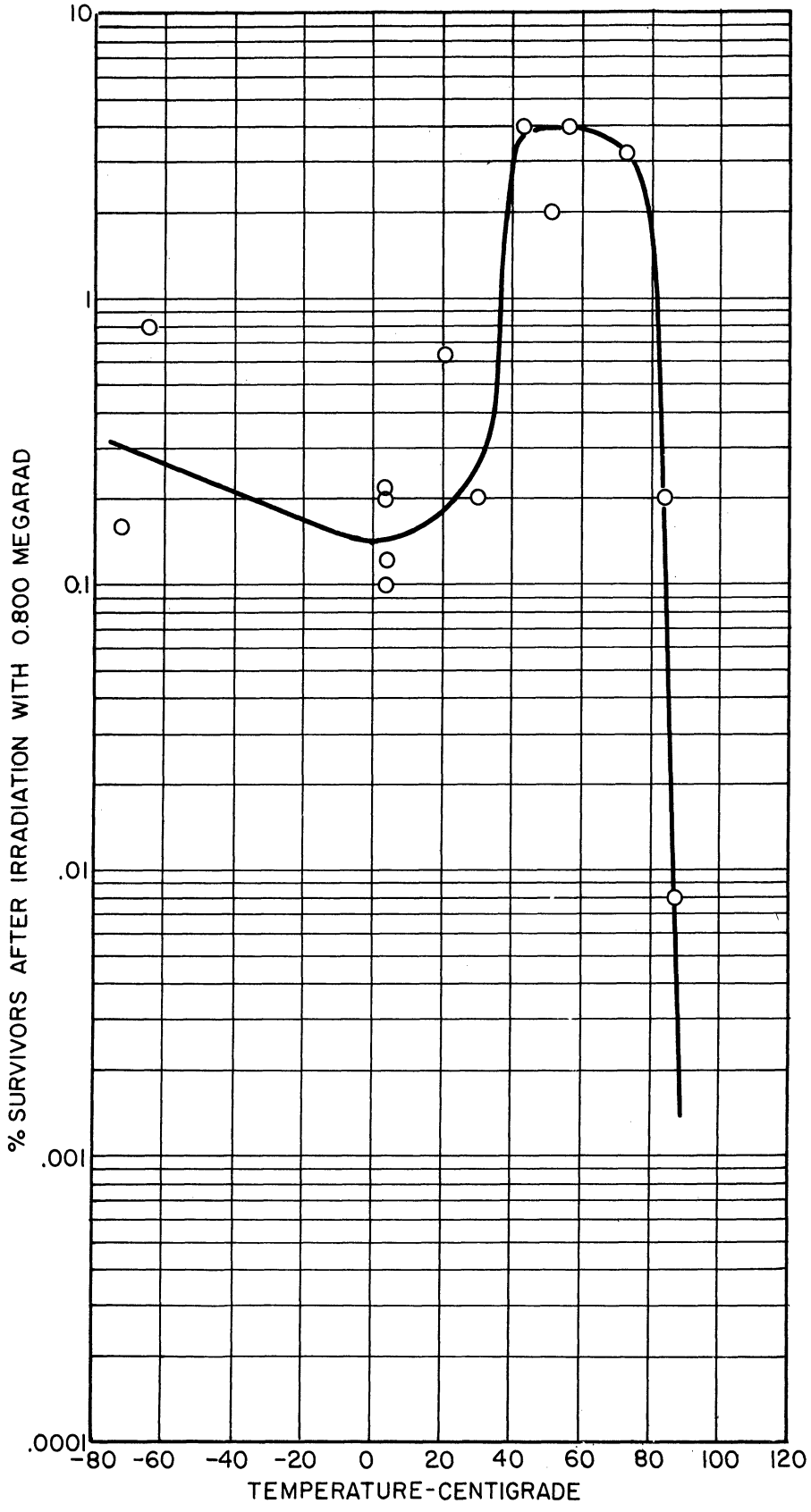

Fig. IV-1. Effect of temperature during irradiation on the lethality of gamma radiation from cobalt-60 for the spores of C. botulinum 62A when they are suspended in M/15 phosphate buffer at pH 7.0 and are irradiated at the rate of 0.203 megarad per hour.

TABLE IV-1

EFFECT OF TEMPERATURE DURING IRRADIATION ON THE LETHALITY OF GAMMA RADIATION FROM COBALT-60 FOR C. BOTULINUM 62A SPORES WHEN SUSPENDED IN M/15 PHOSPHATE BUFFER AT pH 7.0 AND IRRADIATED AT 0.203 MEGARAD PER HOUR

Run No.	Temperature, C°	Initial Inoculum, Millions Per ml	% Survivors at 0.8 Megarad	Log % Survivors at 0.8 Megarad
T-2	-72	0.660	0.16	-0.80
T-12	-65	4.750	0.80	-0.10
I-2*	4	1.900	0.12	-0.90
I-9*	4	1.580	0.22	-0.65
T-5	4	7.150	0.20	-0.70
T-1	4	1.400	0.10	-1.00
T-3	20	0.785	0.63	-0.20
T-8	31	0.725	0.20	-0.70
T-6	43	5.350	4.0	+0.60
T-7	53	7.600	2.0	+0.30
T-4	58	3.550	4.0	+0.60
T-10	73	5.750	3.2	+0.50
T-11	84	4.900	0.20	-0.70
T-9	87	4.350	0.008	-2.10

* Irradiation intensity 0.193 megarad per hour

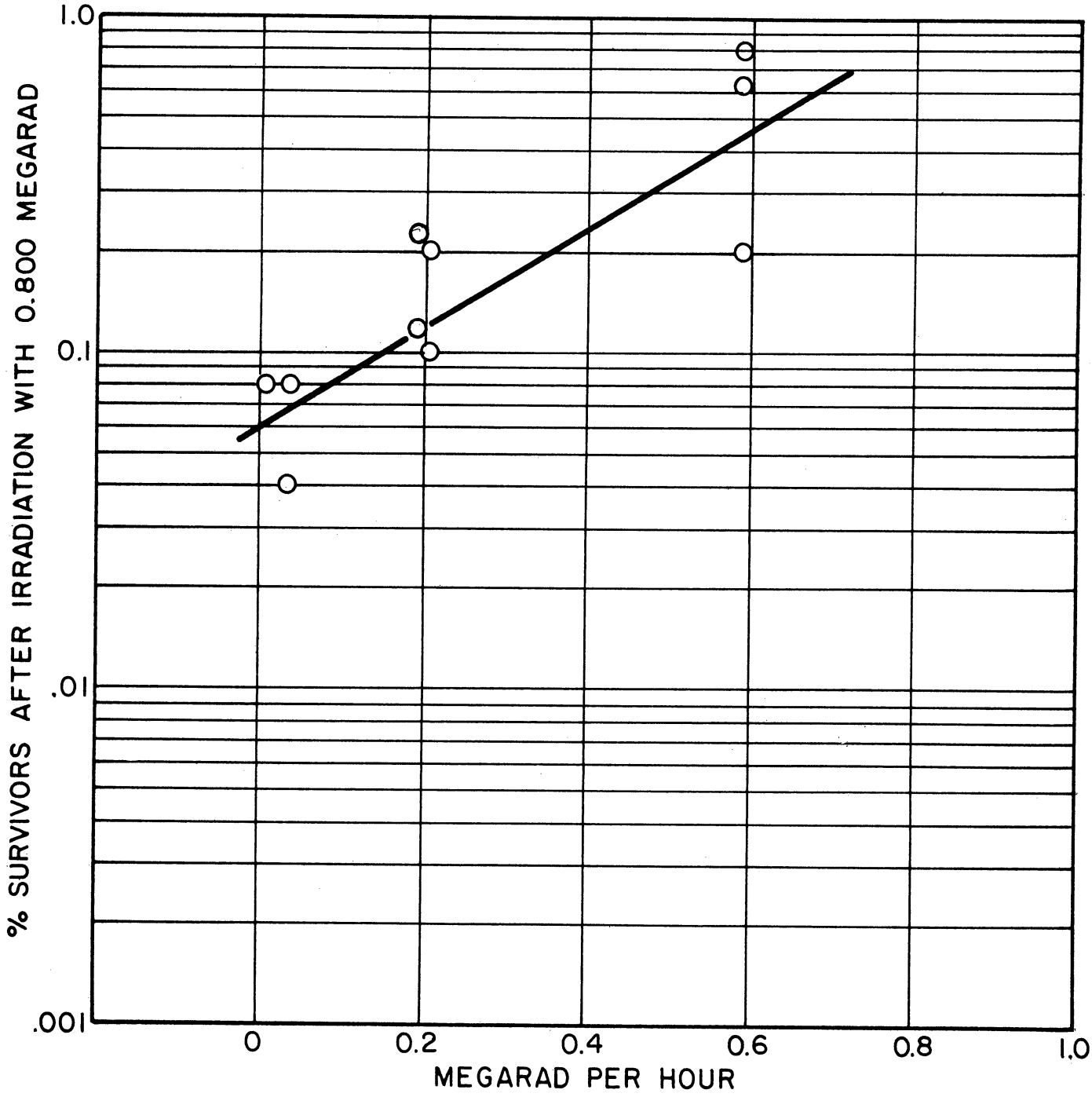

Fig. IV-2. Effect of intensity of irradiation on the lethality of gamma radiation from cobalt-60 for the spores of C. botulinum 62A when they are suspended in M/15 phosphate buffer at pH 7.0 and are irradiated at 4°C.

TABLE IV-2

EFFECT OF INTENSITY OF IRRADIATION ON THE LETHALITY OF GAMMA RADIATION FROM COBALT-60 FOR THE SPORES OF CLOSTRIDIUM BOTULINUM 62A WHEN THEY ARE SUSPENDED IN M/15 PHOSPHATE BUFFER AT pH 7.0 AND ARE IRRADIATED AT 4°C

Run No.	Intensity Megarad Per/hr	Initial Spore Inoculum, Millions per ml	% Survivors at 0.8 Megarad	Log % Survivors at 0.8 Megarad
I-4	0.002	1.300	0.08	-1.1
I-6	0.039	1.700	0.08	-1.1
I-8	0.039	1.900	0.04	-1.4
I-2	0.193	1.900	0.12	-0.9
T-5	0.203	7.150	0.20	-0.7
I-9	0.193	1.580	0.22	-0.65
T-1	0.203	1.400	0.10	-1.0
I-10	0.591	0.990	0.80	-0.1
I-11	0.591	1.100	0.63	-0.2
I-7	0.591	4.650	0.2	-0.7

Status of Work.—It was surprising to note a small but possibly significant effect of irradiation intensity on the lethality of gamma radiation for C. botulinum spores. In our opinion this should be followed up with a more detailed study to determine whether this effect is real and, if so, to establish its magnitude. It should be evaluated at higher radiation intensities than those used. Since it seems to be an increasing effect, it could be very important in the more intense sources contemplated for actual use. The same statements are applicable to the temperature study.

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APPENDIX A

This Appendix includes data from inoculated packs of C. botulinum and PA-3679 spores in canned green peas.

TABLE I-3

F_o VALUES REQUIRED TO STERILIZE CANNED GREEN PEAS 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 AND INCUBATED AT 85°F

Run No. CP-1 —	Can Size	-No. 1 Picnic (211 x 400)
	Product	-Green Peas
	Inoculum	-300 PA-3679 spores per can
	Irradiation	-None
	Processing Temperature	-230°F
	Incubation Temperature	-85°F

F_o	Can No.	Days-to-Gas Formation
Inoculated Controls	1	3
	2	3
Noninoculated Controls	3	4
	4	4
Can 1, 3.25	5	-
Can 2, 3.25	6	-
Can 3, 3.25	7	-
	8	7
Can 1, 4.11	9	-
Can 2, 4.11	10	6
Can 3, 4.11	11	12
	12	-
Can 1, 4.91	13	-
Can 2, 4.91	14	-
	15	-
	16	-

Run No. CP-1 (Concluded)

F_o	Can No.	Days-to-Gas Formation
Can 1, 5.97	17	-
Can 2, 5.97	18	-
	19	-
	20	-
Can 1, 6.95	21	-
Can 2, 6.95	22	-
	23	-
	24	-
Can 1, 8.50	25	-
Can 2, 8.50	26	-
	27	-
	28	-
*Can 1, 2.20	29	5
Can 2, 2.20	30	4
	31	5
	32	4
Can 1, 2.95	33	-
Can 2, 2.95	34	5
Can 3, 2.95	35	-
	36	5

Conclusion: Under these conditions, canned green peas were sterilized by an F_o between 4.1 and 4.9.

* Cans 29 through 36 were packed and processed as part of run CP-6.

Run CP-2 -- Can Size -No. 1 Picnic (211 x 400)
Product -Green Peas
Inoculum -300 PA-3679 spores per can
Irradiation -As Indicated
Processing Temperature -Not Heat-Processed
Incubation Temperature -85°F

Megarad	Can No.	Days-to-Gas Formation
Inoculated Controls	1	5
	2	6
Noninoculated Controls	3	7
	4	-
1.302	5	4
	6	3
	7	3
	8	4
1.770	9	-
	10	8
	11	-
	12	-
2.05	13	-
	14	-
	15	-
	16	-
2.42	17	-
	18	-
	19	-
	20	-

Conclusion: Under these conditions, canned green peas were sterilized by between 1.77 and 2.05 megarad of Cobalt-60 gamma radiation.

Run CP-3 -- Can Size -No. 1 Picnic (211 x 400)
Product -Green Peas
Inoculum -300 PA-3679 spores per can
Irradiation -0.465 Megarad
Processing Temperature -230°F
Incubation Temperature -85°F

F_o	Can No.	Days-to-Gas Formation
Inoculated Controls	1	2
	2	2
Noninoculated Controls	3	2
	4	9
Can 1, 6.00	5	-
Can 2, 6.00	6	-
Can 3, 6.00	7	-
	8	-
Can 1, 4.60	9	-
Can 2, 4.60	10	-
Can 3, 4.60	11	-
	12	-
Can 1, 3.40	13	-
Can 2, 3.40	14	-
	15	-
	16	-
Can 1, 2.25	17	-
Can 2, 2.25	18	-
Can 3, 2.25	19	-
	20	-
Can 1, 1.39	25	8
Can 2, 1.39	26	-
Can 3, 1.39	27	-
	28	-
Can 1, 0.74	*29	-
Can 2, 0.74	*30	20
Can 3, 0.74	*31	-
	*32	-

Conclusion: Following 0.465 megarad of gamma radiation, from Cobalt-60, canned green peas were sterilized by an F_o between 1.39 and 2.25.

* Processing temperature reached 234°F.

Run CP-4 -- Can Size -No. 1 Picnic (211x 400)
Product -Green Peas
Inoculum -300 PA-3679 spores per can
Irradiation -0.930 megarad
Processing Temperature -230°F
Incubation Temperature -85°F

F_o	Can No.	Days-to-Gas Formation
Noninoculated Controls	1	3
	2	3
	3	3
	4	4
Inoculated Controls	5	2
	6	2
	7	2
Can 1, 0.69	8	-
Can 2, 0.69	9	-
Can 3, 0.69	10	-
	11	-
Can 1, 1.44	12	-
Can 2, 1.44	13	-
Can 3, 1.44	14	-
	15	-
Can 1, 0.25	16	4
Can 2, 0.25	17	-
Can 3, 0.25	18	-
	19	-
Can 1, 0.49	20	-
Can 2, 0.49	21	-
Can 3, 0.49	22	-
	23	-
Can 1, 0.14	24	4
Can 2, 0.14	25	-
Can 3, 0.14	26	-
	27	-

Conclusion: Following 0.930 megarad of gamma radiation from Cobalt-60, canned green peas were sterilized by an F_o between 0.25 and 0.49

Run CP-5 -- Can Size -No. 1 Picnic (211 x 400)
Product -Green Peas
Inoculum -300 PA-3679 spores per can
Irradiation -0.697 megarad
Processing Temperature -230°F
Incubation Temperature -85°F

F_o	Can No.	Days-to-Gas Formation
Noninoculated Controls	1	-
	2	6
	3	6
	4	4
Inoculated Controls	5	3
	6	3
	7	3
Can 1, 1.10	8	-
Can 2, 1.10	9	-
Can 3, 1.10	10	-
	11	-
Can 1, 0.69	12	-
Can 2, 0.69	13	-
Can 3, 0.69	14	-
	15	-
Can 1, 0.33	16	4
Can 2, 0.33	17	6
Can 3, 0.33	18	-
	19	6
Can 1, 0.18	20	-
Can 2, 0.18	21	6
	22	6
	23	-

Conclusion: Following 0.697 megarad of gamma radiation from Cobalt-60, canned green peas were sterilized by an F_o between 0.33 and 0.69.

Run CP-6 -- Can Size -No. 1 Picnic (211 x 400)
Product -Green Peas
Inoculum -300 PA-3679 spores per can
Irradiation -0.279 megarad
Processing Temperature -230°F
Incubation Temperature -85°F

F_o	Can No.	Days-to-Gas Formation
Noninoculated Controls	1	3
	2	-
	3	23
	4	6
Inoculated Controls	5	3
	6	3
	7	6
	8	7
Can 1, 3.16	9	-
Can 2, 3.16	10	-
	11	-
	12	-
Can 1, 0.77	13	4
Can 2, 0.77	14	4
Can 3, 0.77	15	4
	16	4
Can 1, 1.47	17	5
Can 2, 1.47	18	4
	19	4
	20	5
Can 1, 2.19	21	-
Can 2, 2.19	22	5
Can 3, 2.19	23	-
	24	-

Conclusion: Following 0.279 megarad of gamma radiation from Cobalt-60, canned peas were sterilized by an F_o between 2.2 and 3.2.

TABLE I-4

F_o VALUES REQUIRED TO STERILIZE CANNED GREEN PEAS 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 AND INCUBATION AT 85°F

Run No.:	PB-1
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	None
Processing Temperature:	230°F
Incubation:	85°F

F_o	Can No.	Days-to-Gas Formation	*Toxin
Inoculated controls	1	2	
	2	2	
Noninoculated controls	1	3	
	2	3	
Can 1, 1.03	5	9	2/2
Can 2, 1.03	6	11	2/2
Can 3, 1.03	7	10	2/2
	8	12	2/2
Can 1, 0.63	9	10	
Can 2, 0.63	10	10	
Can 3, 0.63	11	10	
	12	9	
Can 1, 0.52	13	8	
Can 2, 0.52	14	9	
	15	9	
	16	9	
Can 1, 0.29	17	5	
Can 2, 0.29	18	9	
	19	6	
	20	9	
Can 1, 0.26	21	5	2/2
Can 2, 0.26	22	5	2/2
	23	6	2/2
	24	6	2/2
Can 1, 1.44	25	24	
	26	13	2/2
	27	24	
	28	13	2/2

*Toxin determined by intraperitoneal injection of 0.5 ml of juice into mice (2/2 means 2 dead mice out of 2 injected).

Conclusion: An F_o in excess of 1.44 is required to sterilize the canned peas.

Run No.:	PB-2
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	As Indicated
Processing Temperature:	None
Incubation Temperature:	85°F

Megarad	Can No.	Days-to-Gas Formation	Toxin
1.530	13	6	
	14	9	
	15	9	
	16	9	
2.045	9	-	
	10	8	
	11	16	0/2
	12	-	
3.080	1	-	
	2	-	
	3	-	
	4	-	
3.600	5	-	
	6	-	
	7	17	2/2
	8	17	2/2

Conclusion: Provided that no error was made in recording the radiation dosage for cans 1 through 8, it appears that more than 3.60 megarad are needed for sterilization. It seems more likely that the dosage is between 3.08 and 3.60 megarad, however.

Note: Same controls as used for Run PB-1.

Run No.: PB-3
Can Size: No. 1 Picnic (211 x 400)
Product: Green Peas
Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
Irradiation: 0.465 megarad
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation	Toxin
Can 1, 1.53	1	-	
Can 2, 1.53	2	-	
Can 3, 1.53	3	-	
	4	-	
	5	11	0/2
Can 1, 1.03	6	11	0/2
Can 2, 1.03	7	15	2/2
Can 3, 1.03	8	15	2/2
Can 1, 0.70	9	16	
Can 2, 0.70	10	20	
Can 3, 0.70	11	13	2/2
	12	26	
Can 1, 0.46	13	9	
Can 2, 0.46	14	17	
	15	13	
	16	9	
Can 1, 0.26	17	9	
Can 2, 0.26	18	13	
	19	13	
	20	9	

Conclusion: Under these conditions, canned green peas were sterilized by 0.465 megarad of gamma radiation followed with an F_o between 1.03 and 1.53.

Run No.: PB-4
Can Size: No. 1 Picnic (211 x 400)
Product: Green Peas
Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation	Toxin
Can 1, 1.82	1	-	
Can 2, 1.82	2	-	
	3	-	
	4	-	
Can 1, 1.10	5	12	2/2
	6	13	
	7	10	
	8	15	
Can 1, 1.39	9	15	2/2
Can 2, 1.39	10	-	
	11	-	
	12	12	2/2

Conclusion: Under these conditions, canned green peas were sterilized by F_o values between 1.39 and 1.82.

Run No.:	PB-5
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spore Per Can
Irradiation:	0.930 megarad
Processing Temperature:	230°F
Incubation Temperature:	85°F

F_o	Can No.	Days-to-Gas Formation	Toxin
Can 1, 1.06	1	-	
Can 2, 1.06	2	-	
Can 3, 1.06	3	-	
	4	-	
Can 1, 0.86	5	-	
Can 2, 0.86	6	-	
Can 3, 0.86	7	-	
	8	-	
Can 1, 0.36	9	17	
Can 2, 0.36	10	20	
Can 3, 0.36	11	19	
	12	17	
Can 1, 0.50	13	-	
Can 2, 0.50	14	-	
Can 3, 0.50	15	-	
	16	-	

Conclusion: Under these conditions, canned green peas were sterilized by 0.930 megarad of gamma radiation followed with a F_o between 0.36 and 0.50.

Run No.:	PB-6
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	As Indicated
Processing Temperature:	Not Heat-Processed
Incubation Temperature:	85°F

Megarad	Can No.	Days-to-Gas Formation
2.330	1	12
	2	18
	3	12
	4	19
2.790	5	-
	6	-
	7	-
	8	-
1.980	9	12
	10	17
	11	18
	12	10

Conclusion: Under these conditions, canned green peas were sterilized by between 2.33 and 2.79 megarad of gamma radiation.

Run No.:	PB-7
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	None
Irradiation:	None
Processing Temperature:	230°F
Incubation Temperature:	85°F
Object:	To determine the F_o required to sterilize canned green peas. The peas were frozen green peas purchased from The University of Michigan food stores.

F_o	Can No.	Days-to-Gas Formation	Toxin
Can 1, 0.03	1	4	
Can 2, 0.03	2	5	
Can 3, 0.03	3	4	
	4	5	
Can 1, 0.06	5	4	0/2
Can 2, 0.06	6	6	
Can 3, 0.06	7	4	
	8	-	
Noninoculated, unheated control	9	3	0/2
Inoculated unheated control	10	3	2/2
Irradiated control, 0.465 megarad	11	11	
	12	11	

Conclusion: Canned green peas contain sufficient anaerobic bacterial spores in the frozen condition, as received by us, to require an F_o greater than 0.06 or irradiation with more than 0.465 megarad, to produce sterile peas.

Run No.:	PB-8
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	1.395 Megarad
Processing Temperature:	230°F
Incubation Temperature:	85°F

F_o	Can No.	Days-to-Gas Formation
Can 1, 0.48	9	-
Can 2, 0.48	10	-
Can 3, 0.48	11	-
	12	-
Can 1, 0.33	13	-
Can 2, 0.33	14	-
Can 3, 0.33	15	*32
	16	-
Can 1, 0.21	17	25
Can 2, 0.21	18	25
	19	30
	20	27
Can 1, 0.12	21	27
Can 2, 0.12	22	30
	23	25
	24	25
Can 1, 0.04	25	19
Can 2, 0.04	26	13
	27	18
	28	13

Conclusion: Under these conditions, canned green peas were sterilized by 1.395 megarad of gamma radiation followed with F_o between 0.33 and 0.48.

*Positive for Type B Botulinum toxin.

Run No.:	PB-9
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	None
Irradiation:	None
Processing Temperature:	230°F
Incubation Temperature:	85°F
Object:	Same as Run No. PB-7, to determine the F_o required to sterilize noninoculated peas that we used in our experiments.

F_o	Can No.	Days-to-Gas Formation	Toxin
Can 1, 0.027	1	3	
Can 2, 0.027	2	5	
	3	5	
	4	5	
Can 1, 0.036	5	5	
Can 2, 0.036	6	5	
	7	3	
	8	3	
Can 1, 0.036	9	5	
Can 2, 0.036	10	5	
	11	11	
	12	5	0/4
Can 1, 0.054	13	5	4/4
Can 2, 0.054	14	5	*4/4
	15	3	1/4
	16	-	

*Tests as Botulinum toxin type B with antiserum neutralization.

Conclusion: An F_o in excess of 0.054 is needed to sterilize the non-inoculated canned green peas that we used in our experiments.

Run No.:	PB-10
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	As Indicated
Processing Temperature:	None
Incubation Temperature:	85°F

Megarad	Can No.	Days-to-Gas Formation
1.860	1	12
	2	11
	3	11
0.930	5	10
	6	7
	7	10
2.790	9	-
	10	22
	11	-

Conclusion: Under these conditions, slightly more than 2.790 megarad of gamma radiation are required to sterilize canned green peas.

Run No.:	PB-11
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	None
Processing Temperature:	230°F
Incubation Temperature:	85°F

F_o	Can No.	Days-to-Gas Formation
Can 1, 2.03	5	-
Can 2, 2.03	6	-
	7	-
	8	-
Can 1, 1.54	1	-
Can 2, 1.54	2	-
	3	-
	4	-

Conclusion: Under these conditions, canned green peas require an F_o less than 1.54 to produce sterility.

Run No.:	PB-12
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	None
Irradiation:	None
Processing Temperature:	230°F
Incubation Temperature:	85°F
Object:	Same as Runs PB-7 and PB-9, to determine the F_o required to sterilize noninoculated peas that we used in our experiments.

F_o	Can No.	Days-to-Gas Formation
Can 1, 0.18	1	49
Can 2, 0.18	2	19
	3	5
	4	3
Can 1, 0.31	5	12
Can 2, 0.31	6	12
	7	16
	8	16
Can 1, 0.17	9	15
Can 2, 0.17	10	7
	11	7
	12	46
Can 1, 0.35	13	-
Can 2, 0.35	14	-
	15	12
	16	16
Can 1, 0.52	17	-
Can 2, 0.52	18	-
	19	-
	20	-
Can 1, 0.76	21	-
Can 2, 0.76	22	-
	23	-
	24	-
Can 1, 1.22	25	-
Can 2, 1.22	26	-
	27	-
	28	-

Conclusion: Under these conditions, canned green peas were sterilized by an F_o between 0.35 and 0.52. This represents a rather considerable resistance to heat-processing by the noninoculated (but naturally contaminated) peas which we used in these experiments.

Run No.:	PB-13
Can Size:	No. 1 Picnic (211 x 400)
Product:	Green Peas
Inoculum:	None
Irradiation:	None
Processing Temperature:	230°F
Incubation Temperature:	85°F
Object:	Same as Runs PB-7, PB-9 and PB-12, to determine the F_o required to sterilize the noninoculated peas we used in our experiments.

F_o	Can No.	Days-to-Gas Formation
Can 1, 0.041	1	3
Can 2, 0.041	2	3
	3	3
	4	3
Can 1, 0.20	5	-
Can 2, 0.20	6	8
	7	11
	8	20

Conclusion: An F_o greater than 0.20 is needed to sterilize the canned peas "as received."

Run No.:	PB-14
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	0.465 Megarad
Processing Temperature:	230°F
Incubation Temperature:	85°F

F_o	Can No.	Days-to-Gas Formation
Can 1, 0.60	1	25
Can 2, 0.60	2	29
Can 3, 0.60	3	18
	4	21
Can 1, 0.75	5	-
Can 2, 0.75	6	-
Can 3, 0.75	7	-
	8	-
Can 1, 1.10	9	-
Can 2, 1.10	10	-
	11	-
	12	-
Can 1, 0.50	13	-
Can 2, 0.50	14	-
Can 3, 0.50	15	21
	16	18

Conclusion: Under these conditions, canned green peas were sterilized by 0.465 megarad of gamma radiation followed with an F_o between 0.60 and 0.75.

Run No.:	PB-15
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	0.650 Megarad
Processing Temperature:	230°F
Incubation Temperature:	85°F

F_o	Can No.	Days-to-Gas Formation
Can 1, 0.92	1	-
Can 2, 0.92	2	-
	3	-
	4	-
Can 1, 1.12	5	-
Can 2, 1.12	6	-
	7	-
	8	-
Can 1, 0.80	9	22
Can 2, 0.80	10	-
	11	-
	12	-
Controls:		
Can 1, 0.50	13	8
Can 2, 0.50	14	8
Inoculated,	15	8
nonirradiated	16	8
Can 1, 1.10	17	-
Can 2, 1.10	18	-
Noninoculated,	19	-
nonirradiated	20	-
Can 1, 0.10	21	4
Can 2, 0.10	22	4
Noninoculated,	23	4
nonirradiated	24	4
Can 1, 0.39	25	8
Can 2, 0.39	26	8
Inoculated,	27	8
nonirradiated	28	8
Irradiated only	1	4
(0.650 megarad)	2	-
Inoculated	1	4
Noninoculated	1	4

Conclusions: (a) Under these conditions, canned green peas were sterilized by 0.650 megarad of gamma radiation followed by an F_o between 0.80 and 0.92. (b) The nonirradiated, noninoculated control cans required an F_o between 0.10 and 1.10 for sterilization. (c) An irradiation dose in excess of 0.650 megarad was needed to sterilize the noninoculated canned peas.

Run No.: PB-17
Product: Green Peas
Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
Irradiation: 1.96 Megarad
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
Noninoculated control	a	24
	b	-
Inoculated control	A	6
Can 1, 0.33	1	-
Can 2, 0.33	2	-
Can 3, 0.33	3	-
	4	-
Can 1, 0.22	5	-
Can 2, 0.22	6	-
Can 3, 0.22	7	-
	8	-
Can 1, 0.11	9	-
Can 2, 0.11	10	-
Can 3, 0.11	11	-
	12	-
Can 1, 0.06	13	-
Can 2, 0.06	14	-
Can 3, 0.06	15	-
	16	-

Conclusion: Under these conditions, and after one month's incubation, canned green peas were sterilized by 1.96 megarad of gamma radiation followed by an F_o less than 0.06.

Run No.:	PB-18
Product:	Green Peas
Inoculum:	5,000,000 C. botulinum 213B Spores Per Can
Irradiation:	1.63 Megarad
Processing Temperature:	230°F
Incubation Temperature:	85°F

F_o	Can No.	Days-to-Gas Formation
Controls—same as on Run No. PB-17		
Can No. 1, 0.64	1	-
Can No. 2, 0.64	2	-
Can No. 3, 0.64	3	-
	4	-
Can No. 1, 0.30	5	-
Can No. 2, 0.30	6	-
Can No. 3, 0.30	7	-
	8	-

Conclusions: Under these conditions, and after one month's incubation, canned green peas were sterilized by 1.63 megarad of gamma radiation followed by an F_o less than 0.30.

Run No.: PB-19
Product: Green Peas
Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
Irradiation: As Indicated
Processing Temperature: None
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
Inoculated control	17	6
Noninoculated controls	NI	4
	NI	4
2.79	1	28
	2	-
	3	28
	4	-
3.29	5	-
	6	-
	7	-
	8	-
3.76	9	-
	10	-
	11	-
	12	-
2.35	13	28
	14	23
	15	23
	16	23

Conclusions: Under these conditions, and after one month's incubation, canned green peas were sterilized by between 2.79 and 3.29 megarad of gamma radiation.

APPENDIX B

TABLE II-1

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 g
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 g
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 g
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 g
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 g
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 g
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 g
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 g
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 g
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 g
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 g
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-3

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 g 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 g 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 g 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 g 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 g 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 g 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-2, Runs C-5, C-6, C-8, and C-11.

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

See Table II-4, Runs S-1 and S-2.

Run No.: A-5
Can Size: Mushroom (202 x 202)
Product: Raw ground beef
Inoculum: 3,330,000 C. botulinum 62A spores per gram
Incubation Temperature: 85°F

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

Megarad	Can No.	Days-to-Gas Formation
3.40	1	-
	2	-
	3	-
	4	-
	5	-
3.70	6	-
	7	-
	8	-
	9	-
	10	-
3.40	11	-
	12	-
	13	-
	14	-
	15	-
4.00×10^6	16	-
	17	-
	18	-
	19	-
	20	-
4.30×10^6	21	-
	22	-
	23	-
	24	-
	25	-
3.1×10^6	26	-
	27	-
	28	-
	29	14
	30	-
3.35×10^6	31	-
	32	-
	33	-
	34	-
	35	-

Megarad	Can No.	Days-to-Gas Formation
3.6×10^6	36	-
	37	-
	38	-
Noninoculated Control	NIC-1	2
	NIC-2	2

Conclusion: Under these conditions, raw ground beef was sterilized with between 3.10 and 3.35 megarad of gamma radiation.

TABLE II-4

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 g 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 g 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 g 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	*227
	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

*Not microbiological.
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 g 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 g
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 g 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	*425
	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	-

*Not microbiological spoilage.

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 21B spores per g 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.

TABLE II-5

COMBINED IRRADIATION-HEAT PROCESS REQUIRED TO STERILIZE CANNED PORK LUNCHEON MEAT INOCULATED WITH ANAEROBIC BACTERIAL SPORES

Run No.: LPA 1
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 300 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.75	5	-
	6	-
	7	-
1.18	1	-
	2	-
	3	-
	4	-
3.33	11	-
	12	-
	13	-
5.44	8	-
	9	-
	10	-
Controls:		
Noninoculated,	N1H 1	-
Unprocessed	N1H 2	-
	N1H 3	-
	N1H 4	-
Inoculated beef not heated	1	-

Conclusion: PA 3679 spores did not grow when inoculated into canned pork luncheon meat even though canned under the vacuum caused by steam exhaustion.

Run No.: LPA 2
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
1.09	5	-
	6	-
	7	-
	8	59
3.20	1	-
	2	-
	3	-
	4	-
5.09	9	-
	10	-
	11	-
	12	-
Controls:	INC 1	98
Nonheated,	INC 2	210
Inoculated	INC 3	-
	INC 4	-

Conclusion: Growth of PA 3679 is not assured under the packing conditions used. However, under these conditions the pork luncheon meat was sterilized with an F_o between 1.1, and 3.2 when sterility is defined by lack of gas production in the cans.

Run No.: LPA 4
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.74	1	-
	2	-
	3	-
	4	-
1.70	5	-
	6	-
	7	-
	8	-
3.29	9	-
	10	-
	11	-
	12	-
8.20	13	-
	14	-
	15	-
	16	-
Controls:		
Noninoculated	N1C 1	19
	N1C 2	26
	N1C 3	-
	N1C 4	-
Inoculated	INC 1	-
	INC 2	-

Conclusion: None.

Run No.: LPA 5
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.86	1	-
	2	-
	3	-
	4	-
1.71	5	-
	6	-
	7	-
	8	-
3.47	9	-
	10	-
	11	-
	12	-
6.52	13	-
	14	-
	15	-
Controls:		
Noninoculated	N1C 1	26
	N1C 2	15
	N1C 3	46
Inoculated	INC 1	11
	INC 2	14
	INC 3	15
	INC 4	34

Conclusion: Under these conditions, pork luncheon meat was sterilized with an F_o of 0.86 or less.

Run No. LPA 6
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: 0.930 megarad
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.53	1	-
	2	-
	3	-
	4	-
0.88	9	-
	10	-
	11	-
	12	-
1.60	13	-
	14	-
	15	-
	16	-
3.12	5	-
	6	-
	7	-
	8	-
Controls:		
Noninoculated	N1C 1	-
Inoculated	INC 1	-

Conclusion: None.

Run No.: LPA 7
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: 1.68 megarad
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.17	13	-
	14	-
	15	-
0.38	9	-
	10	-
	11	-
	12	-
0.82	5	-
	6	-
	7	-
	8	-
1.51	1	-
	2	-
	3	-
	4	-
Controls:		
Noninoculated	N1C 1	6
	N1C 2	8
	N1C 3	8
Inoculated	INC 1	5
	INC 2	5
	INC 3	5
	INC 4	6

Conclusion: Under these conditions, pork luncheon meat was sterilized by an F_o of 0.17 or less following irradiation with 1.68 megarad of gamma radiation.

Run No.: LPA 8
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: As indicated
Processing Temperature: None
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
0.930	9	-
	10	-
	11	-
	12	-
1.86	1	-
	2	-
	3	-
	4	-
2.79	5	-
	6	-
	7	-
	8	-
3.72	13	-
	14	-
	15	-
	16	-
Controls:		
Noninoculated	N1C 1	5
	N1C 2	6
	N1C 3	6
	N1C 4	6
Inoculated	INC 1	5
	INC 2	5
	INC 3	5
	INC 4	6

Conclusion: Under these conditions, pork luncheon meat was sterilized with 0.93 megarad of gamma radiation or less.

Run No.: LPA 9
Can Size. No.1Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
1.11	1	56
	2	-
	3	42
	4	-
3.45	5	-
	6	-
	7	-
	8	-
8.15	9	-
	10	-
	11	-
	12	-
Controls:		
Noninoculated	N1C 1	-
	N1C 2	-
Inoculated	INC 1	12
	INC 2	12
	INC 3	6
	INC 4	6

Conclusion: Under these conditions, pork luncheon meat was sterilized by an F_o between 1.11 and 3.45.

Run No.: LB 1
Can Size: Mushroom (202 x 202)
Product: Pork luncheon meat
Inoculum: 1,530,000 C. botulinum 213B spores per can
Irradiation: As indicated
Processing Temperature: None
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxicity
0.186	16	62	*0/3
	17	54	
	18	66	0/4
	19	74	
0.372	11	75	
	12	68	
	13	87	
	14	82	4/4
	15	110	
0.558	1	110	
	2	110	4/4
	3	69	
	4	-	
	5	84	3/4
	6	115	
0.744	7	115	
	8	85	
	9	72	
	10	121	

*The meat in Can No. 16 had a slightly putrid smell, a slimy surface, and the color of the original pork luncheon meat. It contained Gram positive rods and a few cocci.

Conclusion: Under these conditions, pork luncheon meat was not sterilized by 0.744 megarad of gamma radiation.

APPENDIX C

TABLE III-1

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

(a) 25-g samples used for toxin analysis

Code Designation of Can	Date Irradiated	Can No.	Irradiation Dose, Megarad	Group*	Type of C. botulinum Spores	No. of Spores per Gram of Meat	Can Size
H2.5B	11/12/53	-	2.48	U-	62A	4,000	2
H3.0B	11/12/53	-	2.50	U	62A	4,000	2
H3.5B	11/12/53	1	3.62	S	62A	4,000	2
H3.5B	11/12/53	2	3.62	S	62A	4,000	2
BB3.5	12/11/53	1	3.25	S	213B	40,000	2
BB3.5	12/11/53	2	3.25	S	213B	40,000	2
X2B	11/13/53	1	1.89	U	62A	4	2
X2B	11/13/53	2	1.89	U	62A	4	2
X2.5B	11/13/53	4	2.26	S	62A	4	2
H4B	11/12/53	-	3.79	S	62A	4,000	2
H4.5B	11/12/53	1	4.41	S	62A	4,000	2
H4.5B	11/12/53	2	4.41	S	62A	4,000	2
BO3	1/21/54	1	2.75	U	62A	400	2
BO3	1/21/54	2	2.75	U	62A	400	2
BO3	1/21/54	3	2.75	U	62A	400	2
BO3.5	1/21/54	1	3.29	U	62A	400	2
BO3.5	1/21/54	2	3.29	U	62A	400	2
BM2.5	1/23/54	1	2.31	U	62A	40	2
BM3	1/23/54	1	2.75	U	62A	40	2
BM3	1/23/54	2	2.75	U	62A	40	2
BM3	1/23/54	3	2.75	U	62A	40	2
A3	3/12/54	1	2.60	U	213B	40,000	2
A3	3/12/54	2	2.60	U	213B	40,000	2
A3.5	3/12/54	1	3.06	S	213B	40,000	2
A3.5	3/12/54	2	3.06	S	213B	40,000	2
ANIC	3/12/54	-	0	S	None	0	2
X2.5B	11/13/53	1	2.26	S	62A	4	2
X2.5B	11/13/53	2	2.26	S	62A	4	2
X2.5B	11/13/53	3	2.26	S	62A	4	2
BN3	12/31/53	1	2.76	U	62A	400	2
BN3	12/31/53	2	2.76	U	62A	400	2
B2Z	12/18/53	-	1.72	U	62A	0.4	2
B2.5Z	12/18/53	1	2.37	S	62A	0.4	2
B2.5Z	12/18/53	2	2.37	S	62A	0.4	2
BZ-NIC**	12/18/53	-	0	S	0	0	2
B3B	10/ 5/53	-	2.85	U	62A	40,000	2
B3.5B	10/ 5/53	-	3.28	U	62A	40,000	2
X3.5B	11/13/53	1	3.17	S	62A	4	2
X3.5B	11/13/53	2	3.17	S	62A	4	2
A3	3/12/54	-	2.60	U	213B	40,000	2
C4	2/ 8/58	11	2.14	U	213B	560	M
C4	2/ 8/58	12	2.14	U	213B	560	M
C4	2/ 8/58	14	2.14	U	213B	560	M
C3	1/27/58	2	1.86	U	213B	990	M
C5	2/17/57	6	2.74	U	B	4,900,000	M[a]
C5	2/17/57	10	2.74	U	B	4,900,000	M
PB19[b]	12/31/57	5	3.29	S	B	17,800	P[c]
PB19[b]	12/31/57	6	3.29	S	B	17,800	P
PB19[b]	12/31/57	7	3.29	S	B	17,800	P
PB19[b]	12/31/57	8	3.29	S	B	17,800	P
PB19[b]	12/31/57	9	3.29	S	B	17,800	P
CB15	2/14/56	1	3.18	U	B	17,800	P
CB15	2/14/56	5	3.68	S	B	17,800	P
CB15	2/14/56	6	3.68	S	B	17,800	P
CB15	2/14/56	7	3.68	S	B	17,800	P
CB15	2/14/56	8	3.68	S	B	17,800	P
C2	1/22/58	11	3.29	S	B	8,500	M
C9	3/12/58	6	3.42	U	B	6,600,000	M
C9	3/12/58	7	3.42	U	B	6,600,000	M
C9	3/12/58	9	3.42	U	B	6,600,000	M
C9	3/12/58	10	3.42	U	B	6,600,000	M
C9	3/12/58	12	3.14	U-2[d]	B	6,600,000	M
C9	3/12/58	14	3.14	U-2[d]	B	6,600,000	M
C9	3/12/58	15	3.14	U-2[d]	B	6,600,000	M
S2	3/20/58	8	3.72	S	B	1,700,000	M
S2	3/20/58	9	3.72	S	B	1,700,000	M
C9	3/12/58	3	3.86	S	B	6,600,000	M
C9	3/12/58	4	3.86	S	B	6,600,000	M
C9	3/12/58	5	3.86	S	B	6,600,000	M
C2	1/22/58	15	3.29	S	B	8,600	M
C3	1/27/58	12	2.46	S	B	1,200	M
C3	1/27/58	13	2.46	S	B	1,200	M
C4	2/ 8/58	15	2.14	S	B	570	M
C7	3/ 6/58	1	2.66	U	B	4	M
C7	3/ 6/58	4	2.66	U	B	4	M
C7	3/ 6/58	6	2.36	U[d]	B		
C7	3/ 6/58	8	2.36	U[d]	B		
C7	3/ 6/58	9	2.36	U[d]	B		
C7	3/ 6/58	10	2.36	U[d]	B		

(a) M = mushroom style can (202 x 202)
(b) Peas (not meat)
(c) P = No. 1 picnic tin can (211 x 400)
(d) Second lower radiation level than sterility.

*Group
S - that group of cans in a run receiving the lowest radiation dosage in which all the cans remained unswollen upon incubation at that and all higher levels of irradiation.
U - that group of cans in a run receiving the highest radiation dosage in which one or more of the cans swelled upon incubation. The cans examined, of course, had remained unswollen during storage at room temperature. (U- means less radiation than U.)
**Control

(b) 75-g samples used for toxin analysis

Code Designation of Can	Date Irradiated	Can No.	Irradiation Dose, Megarad	Group*	Type of *C. botulinum* Spores	No. of Spores per Gram of Meat	Can Size
C10	3/29/58	11	2.00	S	B	16.7	[a]M
C10	3/29/58	12	2.00	S	B	16.7	M
C10	3/29/58	15	1.77	U	B	16.7	M
C10	3/29/58	19	1.77	U	B	16.7	M
C10	3/29/58	20	1.77	U	B	16.7	M
S3	3/28/58	1	4.00	S	B	17,000	M
S3	3/28/58	3	4.00	S	B	17,000	M
S3	3/28/58	4	4.00	S	B	17,000	M
S3	3/28/58	8	3.53	U	B	17,000	M
S3	3/28/58	10	3.53	U	B	17,000	M
A6	12/12/58	16	4.00	S+	A	3,330,000	M
A6	12/12/58	17	4.00	S+	A	3,330,000	M
A6	12/12/58	19	4.00	S+	A	3,330,000	M
A6	12/12/58	20	4.00	S+	A	3,330,000	M
AC2	7/23/58	11	3.50	U	A	4,800,000	M
AC2	7/23/58	12	3.50	U	A	4,800,000	M
AC2	7/23/58	20	2.75	U-	A	4,800,000	M
AC2	7/23/58	23	3.00	U-	A	4,800,000	M
A3	7/21/58	11	3.50	U-	A	3,200,000	M
A3	7/21/58	12	3.50	U-	A	3,200,000	M
A3	7/21/58	14	3.50	U-	A	3,200,000	M
AC1	7/11/58	17	3.40	U	A	5,200,000	M
AC1	7/11/58	18	3.40	U	A	5,200,000	M
AC1	7/11/58	20	3.40	U	A	5,200,000	M
A2	7/ 7/58	14	3.20	U	A	2,670,000	M
A2	7/ 7/58	15	3.20	U	A	2,670,000	M
A3	7/21/58	26	3.80	U	A	3,200,000	M
A3	7/21/58	29	3.80	U	A	3,200,000	M
A3	7/21/58	30	3.80	U	A	3,200,000	M
A6	12/12/58	1	3.40	S	A	3,330,000	M
A6	12/12/58	2	3.40	S	A	3,330,000	M
A6	12/12/58	4	3.40	S	A	3,330,000	M
A6	12/12/58	5	3.40	S	A	3,330,000	M
S6	5/ 8/58	16	3.30	S	B	1,440,000	M
S6	5/ 8/58	17	3.30	S	B	1,440,000	M
S6	5/ 8/58	20	3.30	S	B	1,440,000	M
S6	5/ 8/58	21	3.30	S	B	1,440,000	M
AC1	7/11/58	5	4.10	S+	A	5,200,000	M
AC1	7/11/58	8	4.80	S+	A	5,200,000	M
AC1	7/11/58	26	3.85	S	A	5,200,000	M
AC1	7/11/58	28	3.85	S	A	5,200,000	M
A2	7/ 7/58	9	4.90	S+	A	2,670,000	M
A3	7/21/58	6	3.80	S	A	3,200,000	M
A3	7/21/58	7	3.80	S	A	3,200,000	M
A3	7/21/58	10	3.80	S	A	3,200,000	M
A6	12/12/58	7	3.70	S+	A	3,300,000	M
A6	12/12/58	8	3.70	S+	A	3,300,000	M
A6	12/12/58	21	4.30	S+	A	3,300,000	M

[a]M = mushroom style can (202 x 202)

S - that group of cans in a run receiving the lowest radiation dosage in which all the cans remained unswollen upon incubation at that and all higher levels of irradiation. (S+ means more radiation than S.)

U - that group of cans in a run receiving the highest radiation dosage in which one or more of the cans swelled upon incubation. The cans examined, of course, had remained unswollen during storage at room temperature. (U- means less radiation than U.)

TABLE III-2

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

(a) 25-g samples used for toxin analysis

Code Designation of Can	Can No.	Toxicity Test of Meat*	Growth in Liver Broth	Gram Stain of Liver Broth	Toxicity Test of Liver Broth*
H2.5B		0/4	1 Not apparent	1 Few G(+) rods	0/2
			2 Not apparent	2 Few G(+) rods	
			3 Not apparent	3 Few G(+) rods	
H3.0B		0/4	1 Not apparent	1 Few G(+) rods	0/2
			2 Not apparent	2 Few G(+) rods	
			3 Not apparent	3 Few G(+) rods	
H3.5B	1	0/4	1 Not apparent	1 Few G(+) rods	0/2
			2 Not apparent	2 Few G(+) rods	
			3 Not apparent	3 Few G(+) rods	
H3.5B	2	0/4	1 Not apparent	1 0	0/2
			2 Not apparent	2 0	
			3 Not apparent	3 0	
BB3.5	1	0/4	1 Not apparent	1 0	0/2
			2 Not apparent	2 0	
			3 Not apparent	3 0	
BB3.5	2	0/4	1 Not apparent	1 0	0/2
			2 Not apparent	2 0	
			3 Not apparent	3 0	
X2B	1	0/3	1 Not apparent	1 0	
			2 Not apparent	2 0	
			3 Not apparent	3 0	
X2B	2	0/3	1 Not apparent	1 0	
			2 Not apparent	2 0	
			3 Not apparent	3 0	
H4B		0/4	None	G(+) rods	0/4
			?	G(+) cocci; G(-) rods	0/4
			?	G(-) rods (few)	0/4
H4.5B	1	0/4	Neg	G(+) rods	1/4(a)
			?	Nothing	0/4
			?	G(+) cocci	0/4
H4.5B	2	0/4	None	G(+) rods	0/4
			?	G(+) rods	0/4
			?	G(+) rods	0/4
B03	1	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
B03	2	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
B03	3	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
B03.5	1	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
B03.5	2	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
BM2.5	1	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
BM3	1	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
BM3	2	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
BM3	3	0/4	None	Nothing	-
			None	Nothing	-
			Growth	G(+) rods; G(+) cocci	4/4(b)

Code Designation of Can	Can No.	Toxicity Test of Meat	Growth in Liver Broth	Gram Stain of Liver Broth	Toxicity Test of Liver Broth
A3	1	0/4	c	c	c
A3	2	1/4(d)	c	c	c
A3.5	1	0/4	c	c	c
A3.5	2	0/4	c	c	c
A-NIC**		0/4	c	c	c
X2.5B	1	0/4	c	c	c
X2.5B	2	0/4	c	c	c
X2.5B	3	0/4	c	c	c
BN3	1	0/4	c	c	c
BN3	2	0/4	c	c	c
B2Z	1	1/4(e)	c	c	c
B2.5Z	1	0/4	c	c	c
B2.5Z	2	0/4	c	c	c
BZ-NIC**		0/4	c	c	c
B3B		0/4	None	Nothing	-
B3.5B		0/4	None	Nothing	-
X3.5B	1	0/4	None	Nothing	-
X3.5B	2	0/4	None	Nothing	-
C4	11	0/4	None	Nothing	-
C4	12	0/4	None	Nothing	-
C4	14	0/4	None	Nothing	-
C3		1/4(f)	None	Nothing	-
C5(g)	6	0/4	-	-	-
C5(g)	10	0/4	-	-	-
PB19	5	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
PB19	6	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
PB19	7	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
PB19	8	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
PB19	9	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
CB15	1	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
CB15	5	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
CB15	6	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
CB15	7	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
CB15	8	0/4	None	Nothing	-
			None	Nothing	-
			None	Nothing	-
CB2(g)	11	0/4	-	-	-
C9	6	0/4	None	Nothing	0/4
C9	7	0/4	None	Few G(+) cocci rods(h)	0/4
C9	9	0/4	None	Few G(+) cocci rods(h)	0/4
C9	10	0/4	None	Nothing	0/4
C9	12	0/4	None	Few G(+) cocci rods(h)	0/4
C9	14	1/4	None	Nothing	0/4
C9	15	0/4	None	Nothing	0/4
S2	8	0/4	0	0	-
S2	9	0/4	0	0	-
C9	3	0/4	0	Few cocci	-
C9	4	0/4	0	0	-
C9	5	0/4	0	0	-
C2	15	0/4	0	0	-
C3	12	0/4	0	0	0
C3	13	0/4	0	Few cocci	0
C4	15	0/4	0	0	-
C7	1	0/4	0	0	-
C7	4	0/4	0	0	-
C7	6	0/4	0	0	-
C7	8	0/4	0	0	-
C7	9	0/4	0	0	-
C7	10	0/4	0	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.
**Control
(a) Recheck of supernatant using two mice resulted in no deaths, so death not due to toxin.
(b) Antitoxin study was inconclusive.
(c) Improperly prepared media spoiled these data.
(d) Recheck on meat showed 0/4, so concluded no toxin.
(e) Death on third day, due to rectal Hemorrhage—no toxin indicated.
(f) Recheck: results 0/3 mice, so no toxin indicated.
(g) Entire can contents extracted for toxin determination.
(h) Probably organisms carried over from original meat.

Note: All cans in S2, C9, C2, C3, C4, and C7 were tested in parallel with Wynns' medium. Also, after the meat samples were added to the tubes of media, the screw caps of the culture tubes were tightened and the tubes of samples were heat-shocked at 75°C for 20 minutes. Results obtained with this technique were identical with those obtained in the parallel tubes of liver media.

(b) 75-g samples used for toxin analysis

Code Designation of Can	Can No.	Toxicity Test of Meat[a]	Growth in Liver Broth	Gram Stain of Liver Broth	Toxicity Test of Liver Broth
C10	11	0/4	0	0	0/2
C10	12	0/2	0	0	0/2
C10	15	0/4	0	0	0/2
C10	19	0/4	0	0	0/2
C10	20	0/4	0	0	0/2
S3	1	0/4	0	0	0/2
S3	3	0/4	0	0	0/2
S3	4	0/4	0	0	0/2
S3	8	0/4	0	0	0/2
S3	10	0/4	0	0	0/2
A6	16	4/4	0	0	-
A6	17	4/4	0	0	-
A6	19	4/4	0	0	-
A6	20	4/4	0	0	-
AC2	11	4/4	0	0	-
AC2	12	4/4	0	0	-
AC2	20	4/4	0	0	-
AC2	23	4/4	0	0	-
A3	11	4/4	0	0	2/4
A3	12	4/4	0	0	-
A3	14	4/4	0	0	-
AC1	17	4/4	0	0	-
AC1	18	4/4	0	0	-
AC1	20	4/4	0	0	-
AC2	14	4/4	0	0	2/4
A2	15	4/4	0	0	-
A3	26	4/4	0	0	-
A3	29	4/4	0	0	-
A3	30	4/4	0	0	-
A6	1	4/4	0	0	-
A6	2	4/4	0	0	-
A6	4	4/4	0	0	-
A6	5	4/4	0	0	-
S6	16	0/4	0	0	-
S6	17	0/6	0	0	-
S6	20	0/4	0	0	-
S6	21	0/3	0	0	-
AC1	5	3/3	0	0	-
AC1	8	3/3	0	0	-
AC1	26	3/3	0	0	-
AC1	28	4/4	0	0	-
A2	9	4/4	0	0	-
A3	6	3/4	0	0	-
A3	7	4/4	0	0	-
A3	10	4/4	0	0	-
A6	7	4/4	0	0	-
A6	8	4/4	0	0	-
A6	21	4/4	0	0	-

[a] Where positive, the presence of toxin was verified by antitoxin neutralization.
- means not tested.
0 negative results.

APPENDIX D

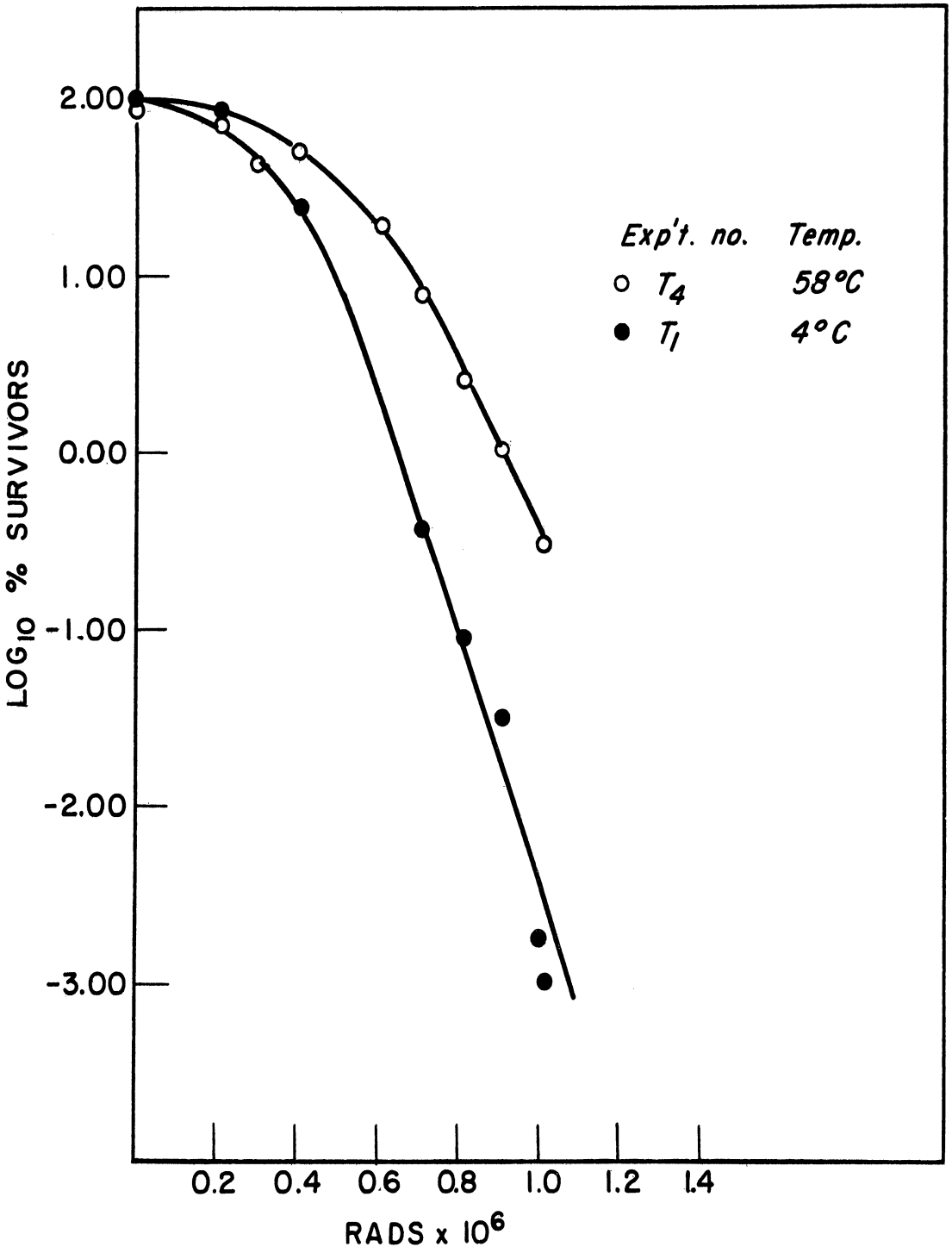

Fig. D1. Effect of temperature during irradiation on the survival of C. botulinum 62A spores.

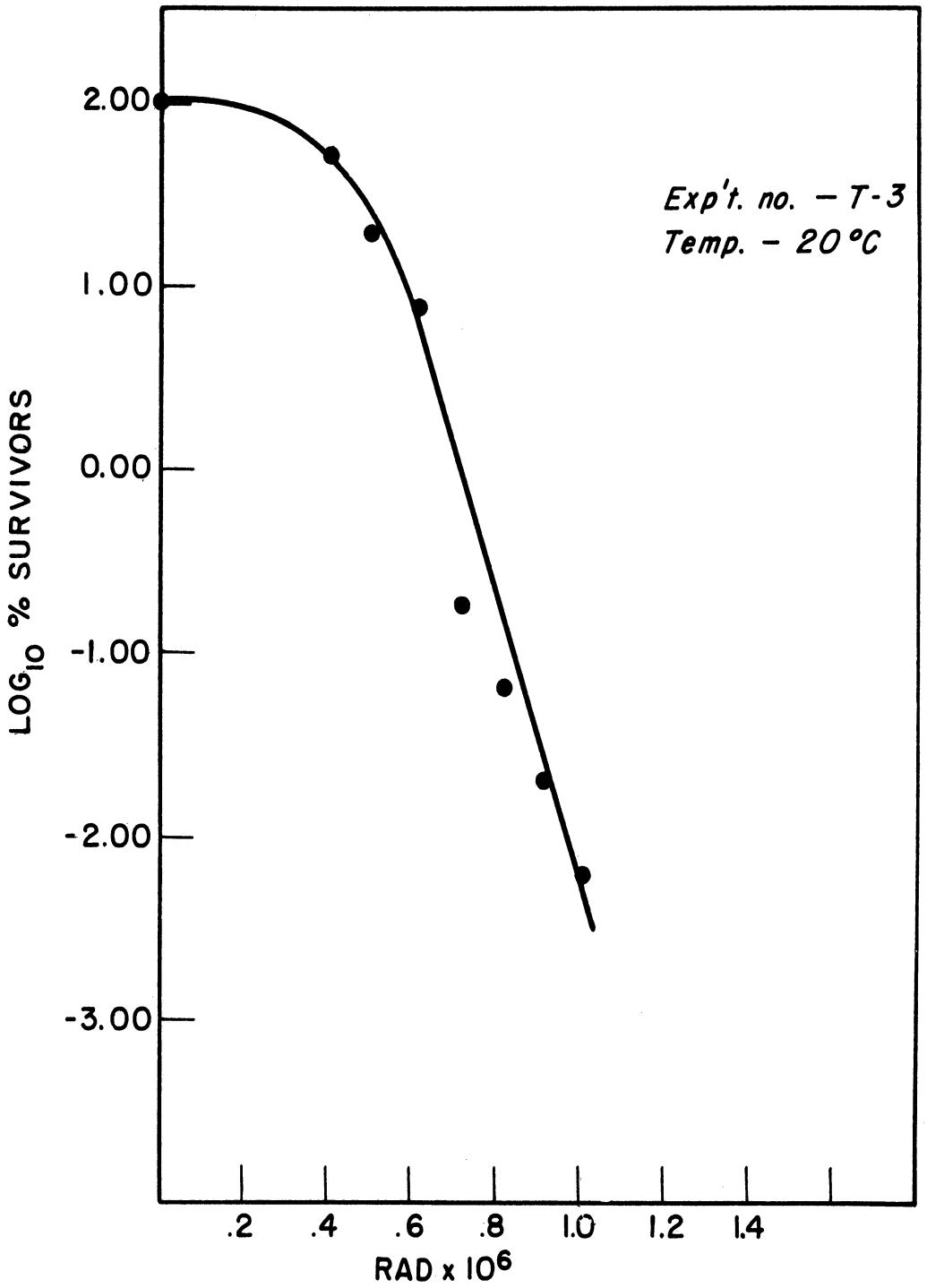

Fig. D2. Effect of temperature during irradiation on the survival of C. botulinum 62A spores.

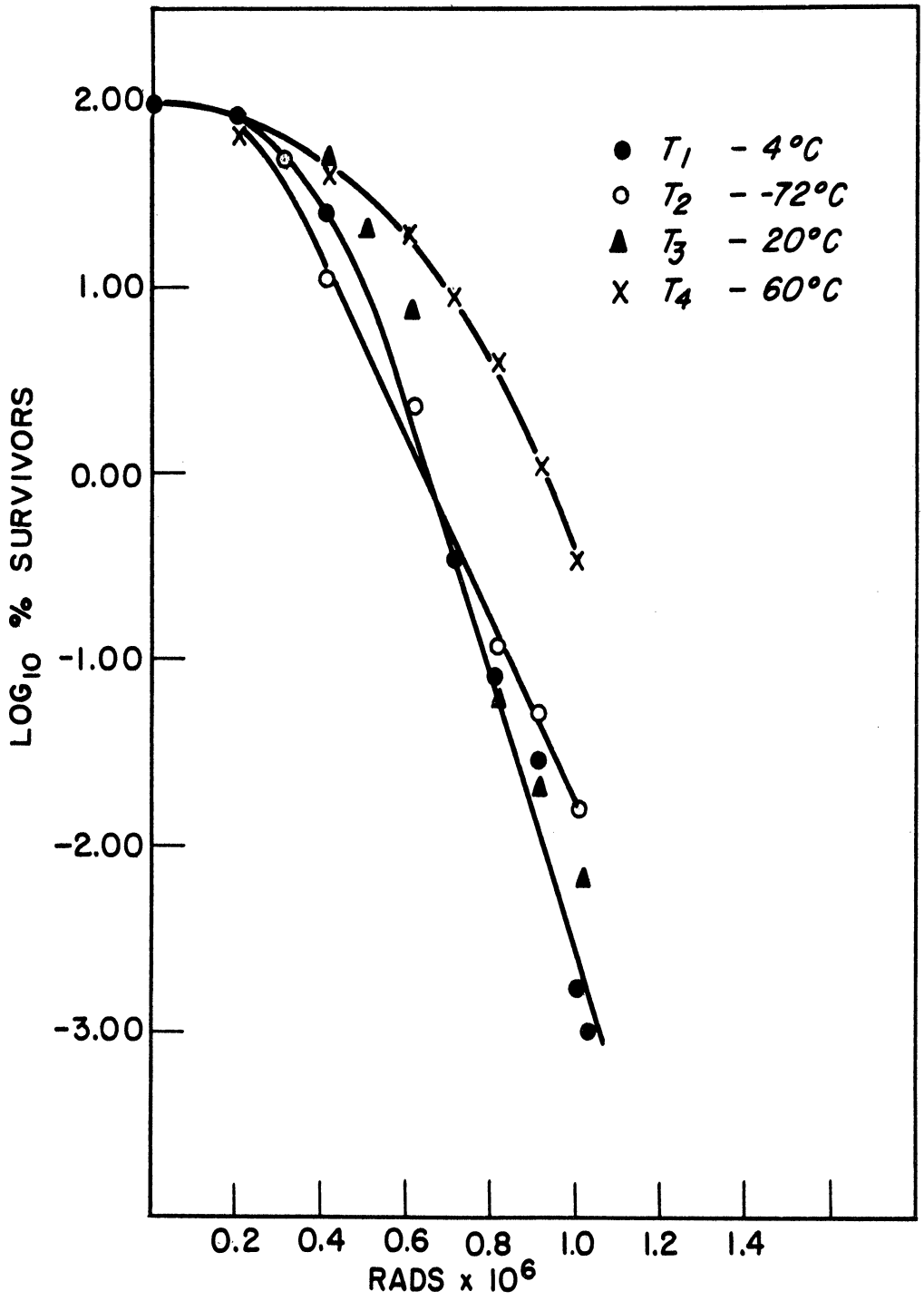

Fig. D3. Effect of temperature during irradiation on the survival of C. botulinum 62A spores.

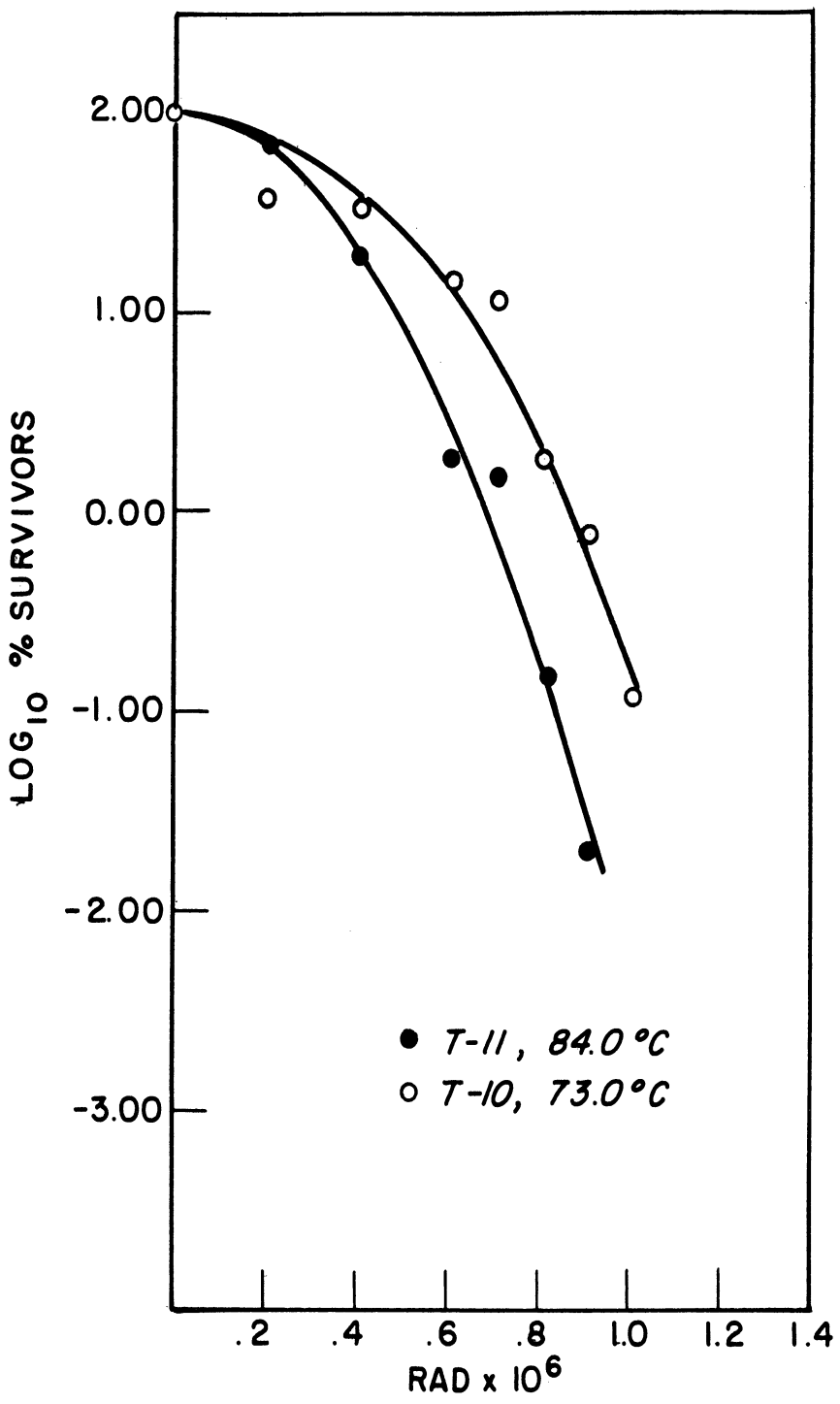

Fig. D4. Effect of temperature during irradiation on the survival of C. botulinum 62A spores.

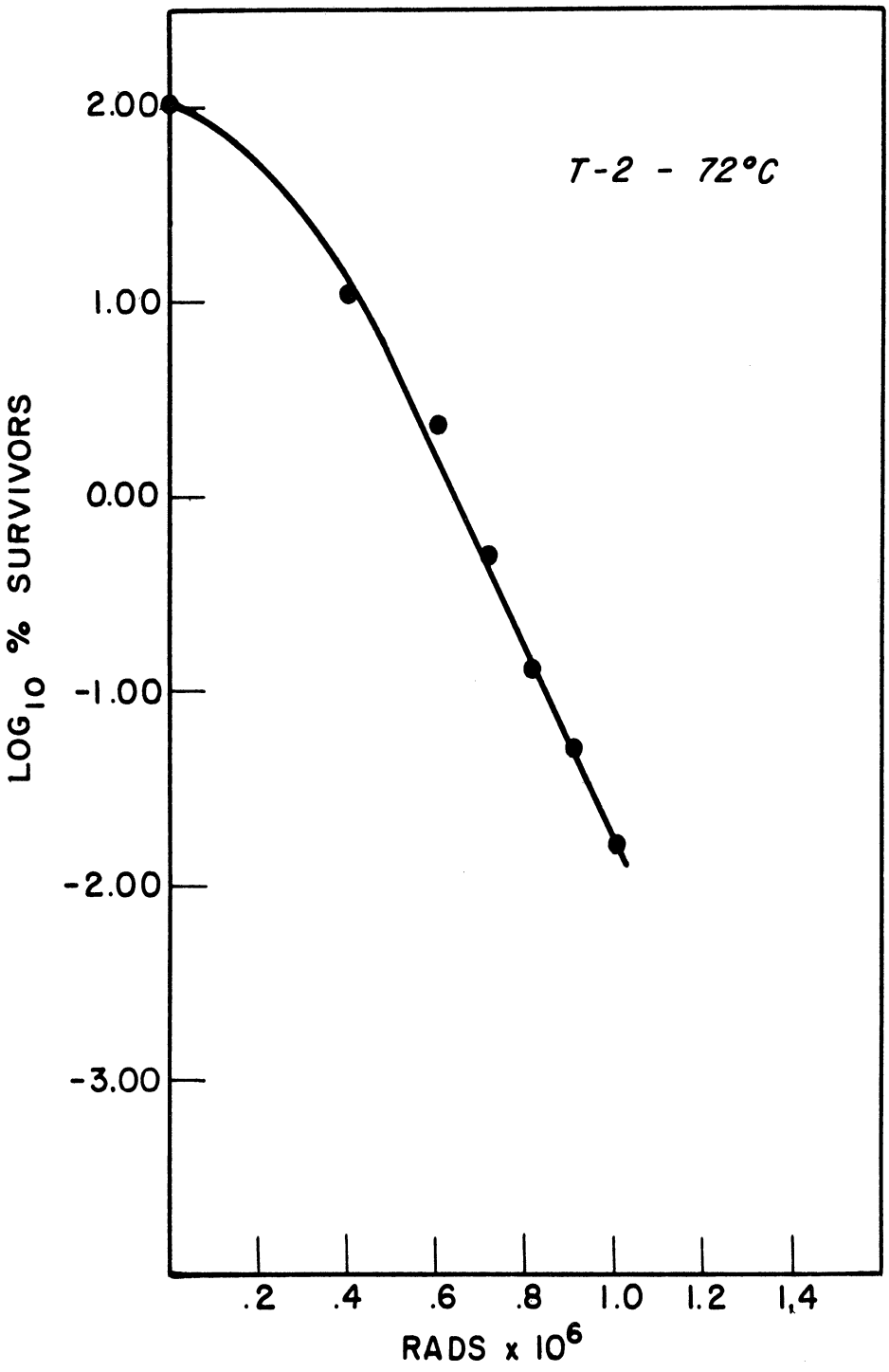

Fig. D5. Effect of temperature during irradiation on the survival of C. botulinum 62A spores.

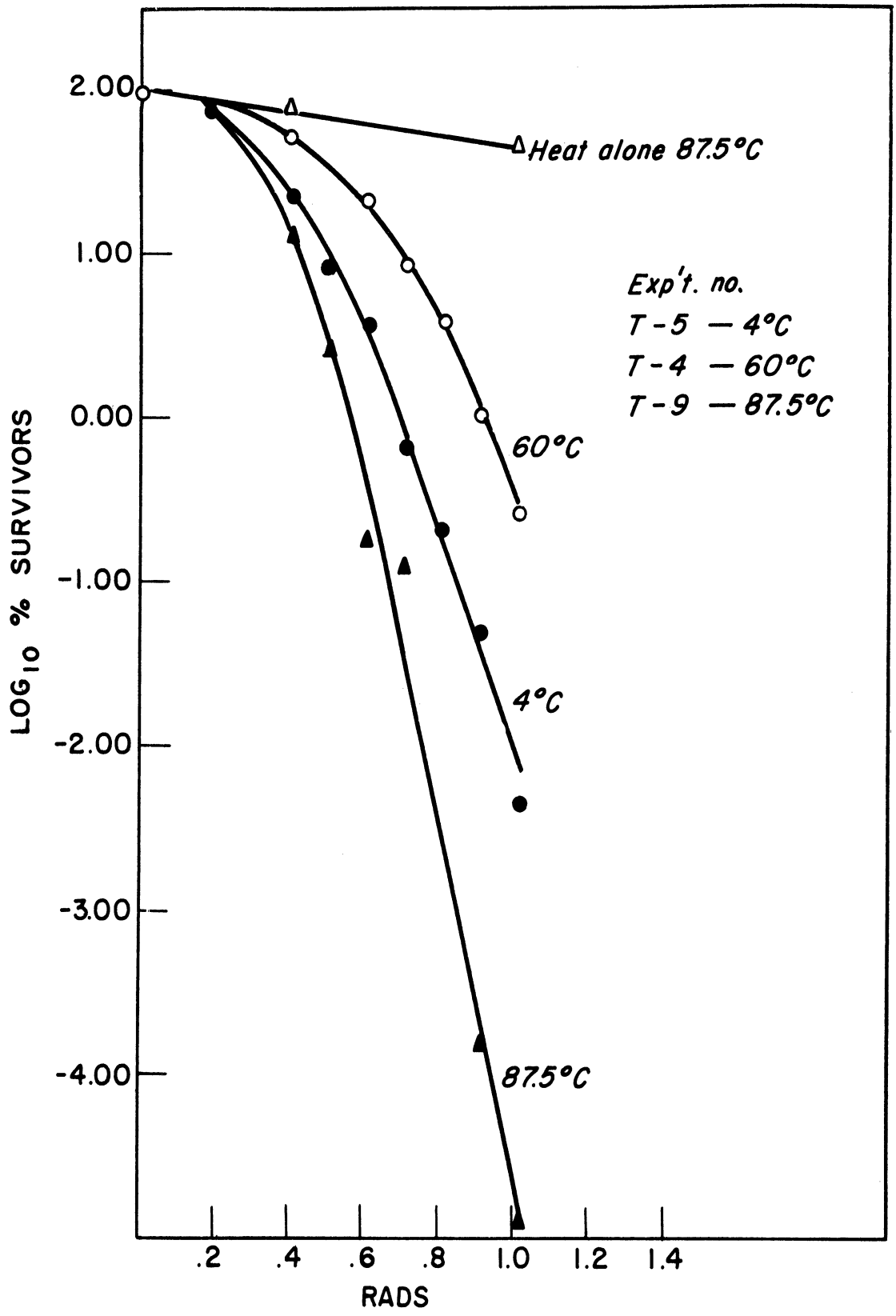

Fig. D6. Effect of temperature during irradiation on the survival of C. botulinum 62A spores.

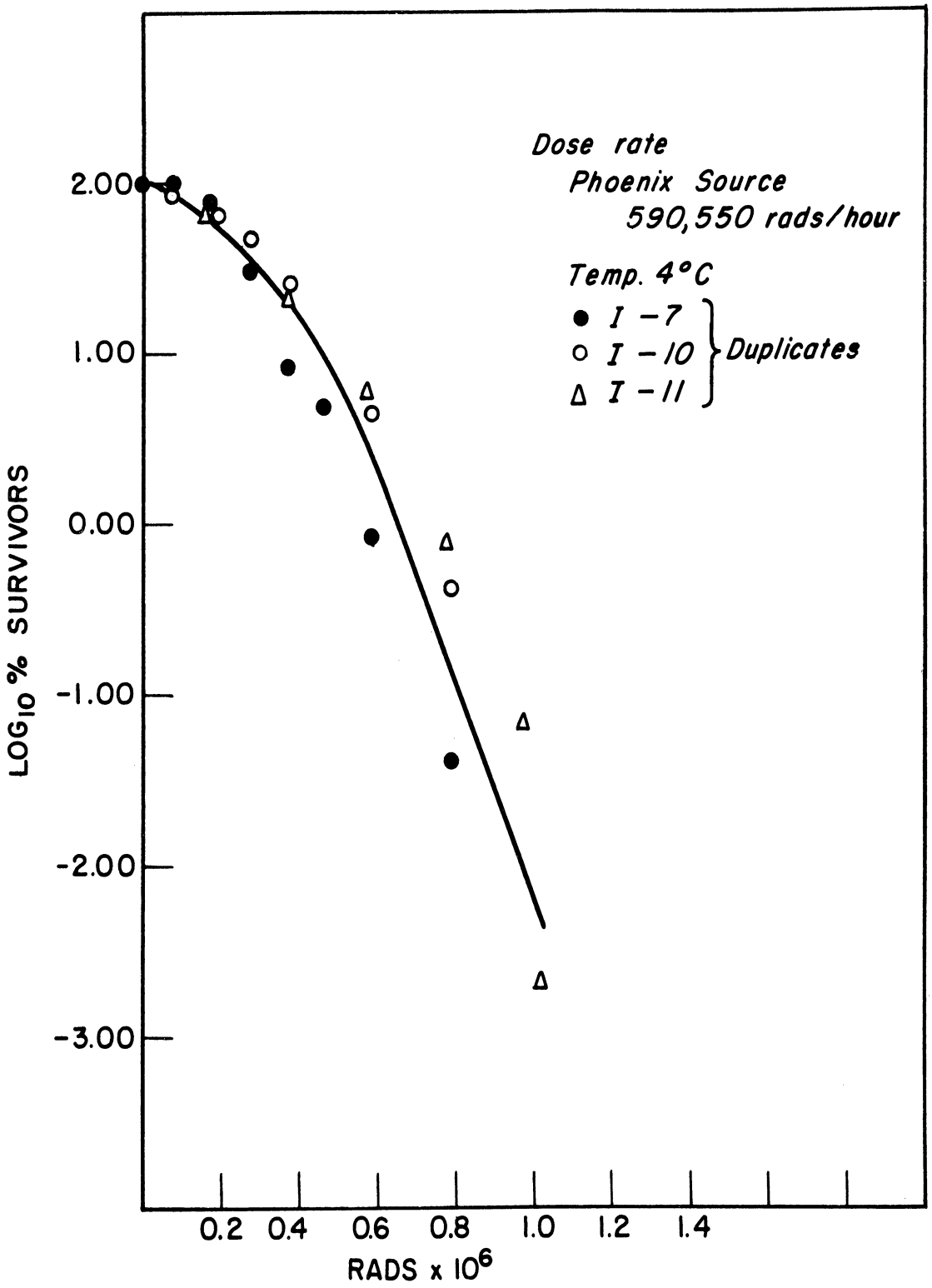

Fig. D7. Effect of variable irradiation intensity from cobalt-60 at constant temperature on the survival of C. botulinum 62A spores.

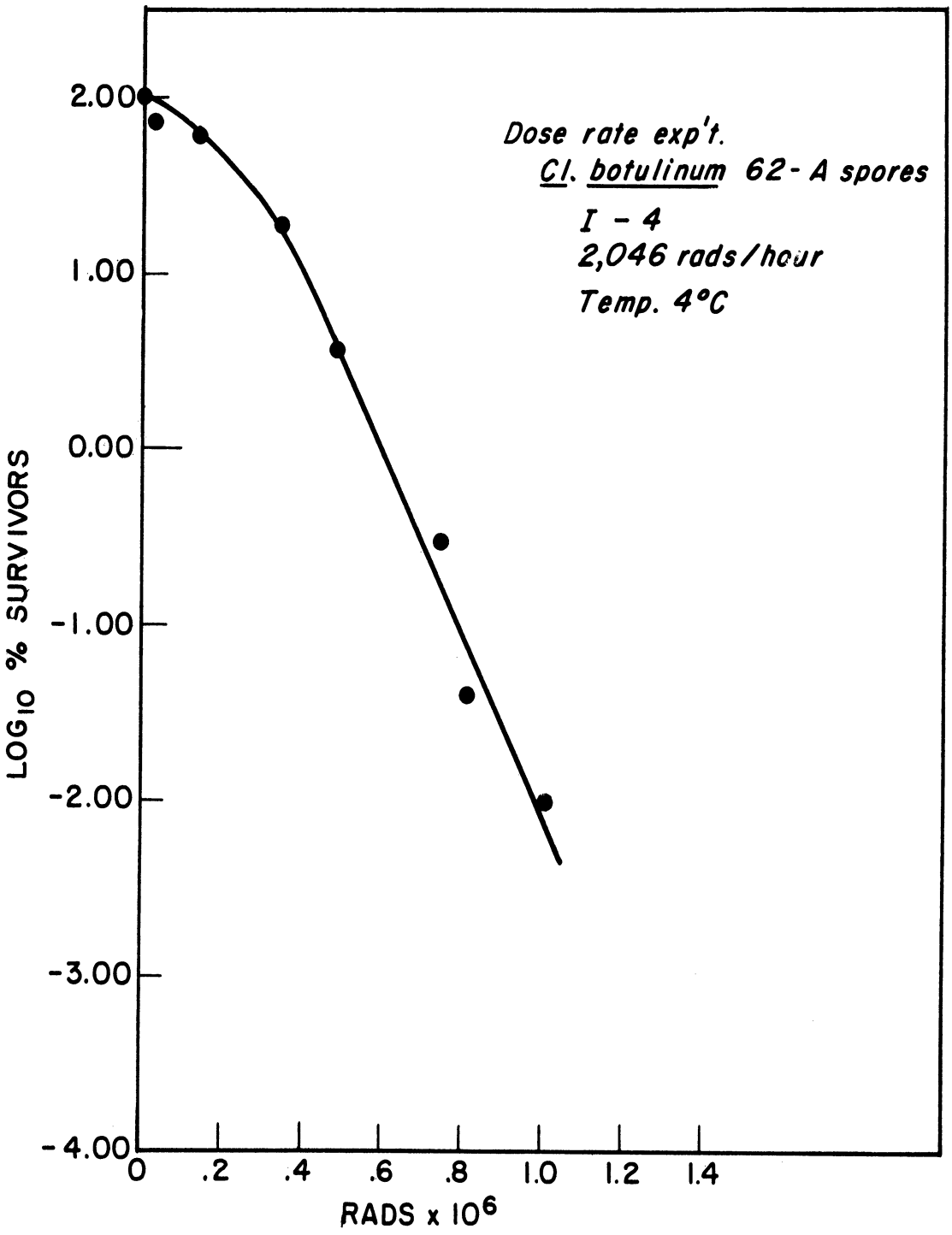

Fig. D8. Effect of variable irradiation intensity from cobalt-60 at constant temperature on the survival of C. botulinum 62A spores.

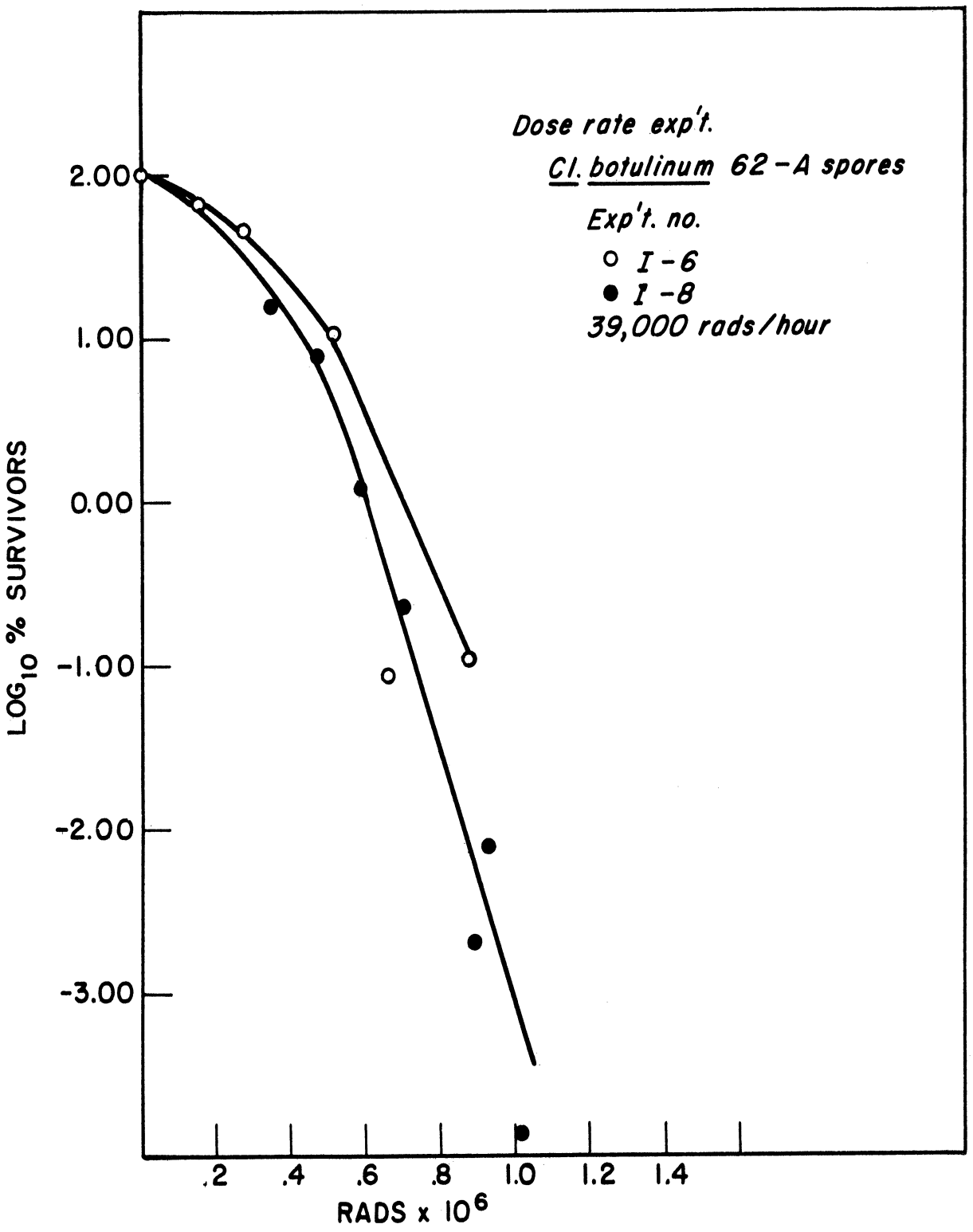

Fig. D9. Effect of variable irradiation intensity from cobalt-60 at constant temperature on the survival of C. botulinum 62A spores.

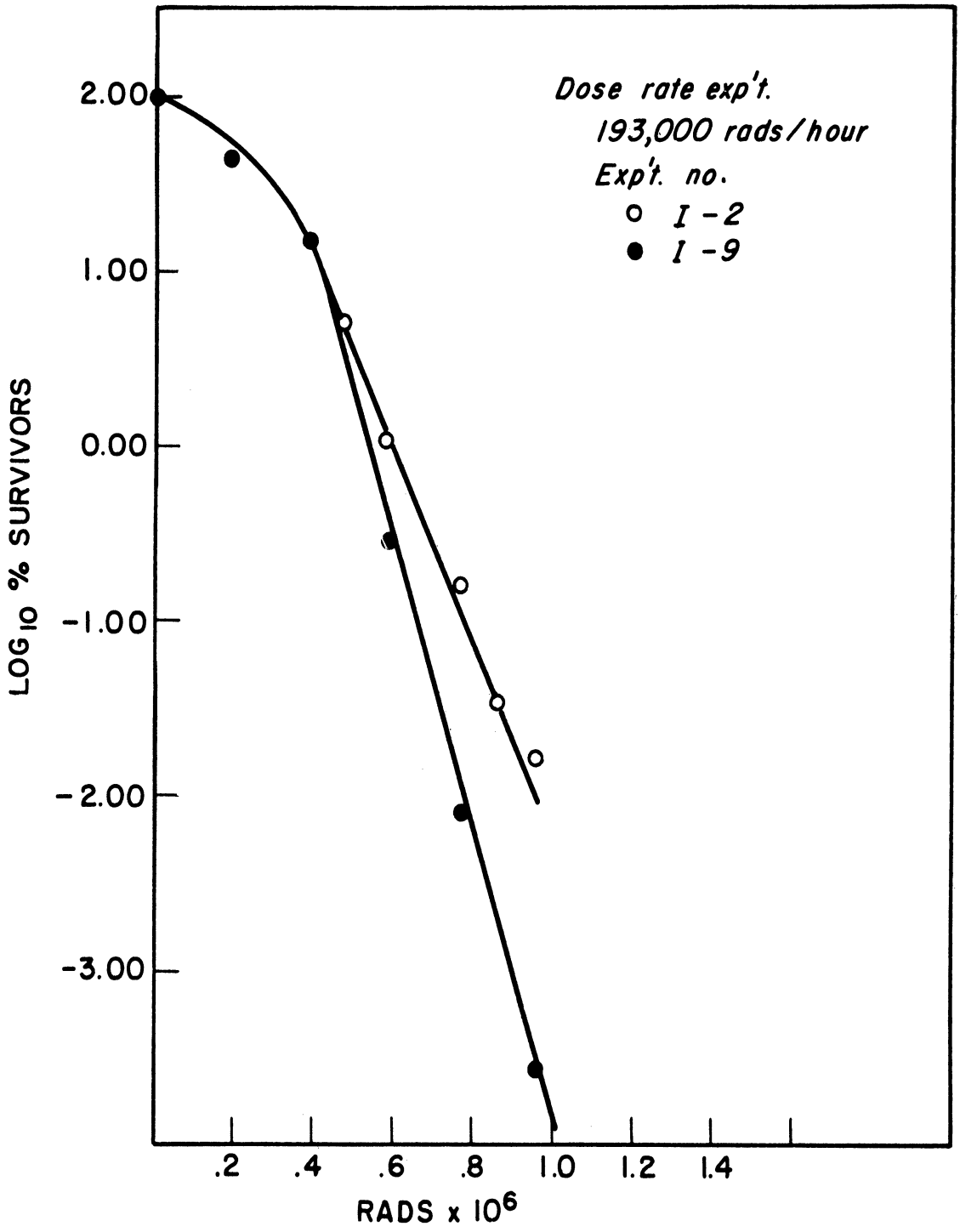

Fig. D10. Effect of variable irradiation intensity from cobalt-60 at constant temperature on the survival of C. botulinum 62A spores.

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Canned ground beef containing 5,000,000 C. botulinum spores per gram were sterilized with between 3.80 and 3.85 megarad of gamma rays. Higher spore inocula would probably require higher sterilization dosages. Botulinus toxin was regularly found in irradiation-sterilized and incubated cans of ground beef inoculated with 2,670,000 or more C. botulinum 62A spores per gram. The lethality of gamma rays for C. botulinum spores was found to be slightly dependent upon both the temperature during irradiation and the intensity of the radiation field. Canned peas, inoculated with C. botulinum spores, were more easily sterilized with combined irradiation-heat processing than by either form of energy alone.

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