ENGINEERING RESEARCH INSTITUTE
THE UNIVERSITY OF MICHIGAN
ANN ARBOR

Final Report

COMBINED USE OF HEAT AND RADIATION TREATMENT FOR STERILIZATION OF FOODS

Period 7 June 1955 to 31 August 1956

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CONTRACT RESEARCH PROJECT REPORT

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GENERAL SUMMARY

In order that irradiation processing of foods can be intelligently considered, basic factual information is needed. Such information includes the effects of temperature on the lethality of ionizing radiations for anaerobic bacterial spores that are important in food sterilization, the effects of medium components, as well as the combined effects of temperature and such radiations. Where it may be planned to use irradiation alone for processing foods, it is necessary to know whether or not such a treatment is effective at refrigerating or freezing temperatures which protect the food before irradiation. Further, do chemical additives used in the preparation of the food affect the sterilizing effectiveness of ionizing radiation? These and other questions have provided the necessity for and objectives of this work.

The work has so far established the following:

1. An induction dosage of approximately one megarep of gamma radiation is required before the combined irradiation—heat processing of

food shows advantage. For example, using 300 C. botulinum 213B spores in a No. 1 picnic can of ground beef, sterility was attained with an F_0 of 0.15 following 1.0 megarep of radiation. But, following 0.5 megarep or less, an F_0 of 0.4 was needed to produce sterile cans of meat.

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

3. Different anaerobic bacterial spores have different temperature ranges in which they are less sensitive to the lethal effects of gamma radiation. However, when such radiations are to be used alone for food sterilization, refrigeration or freezing temperatures are best suited for the process since both C. botulinum and PA 3679 spores are more sensitive to gamma radiation at low temperatures than they are at temperatures between 5°C and 95°C. This is compatible with processing schedules designed to protect foods by refrigeration or freezing before and during irradiation.

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

It is expected that future work will establish whether preirradiation of canned raw meat produces similar reductions in the amount of heat required to sterilize previously irradiated foods. Also, the Z value of irradiated spores of C. botulinum has been assumed to be the same as that of nonirradiated spores. This must be factually established before combined irradiation—heat processing schedules can be developed or even properly evaluated.

Since the present study of the combined irradiation—heat processing of foods has been confined to ground beef, and C. botulinum spores, this work should be extended to include other food products and other anaerobic bacterial spores.

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

1. Combined irradiation—heat sterilization of canned ground beef: by Lloyd L. Kempe, J. T. Graikoski, and P. F. Bonventre.

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

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

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

PHASE I

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

SUMMARY

Canned ground beef, packed in No. 1 picnic tin cans and inoculated with Clostridium botulinum 213B spores, was sterilized using preirradiation and heat processing. Two spore concentrations were tested.

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

b. Using 300 C. botulinum 213B spores per can of meat, sterilization was attained with an F_0 of approximately 0.4, while with irradiation alone, approximately 1.7 megarep of gamma radiation from cobalt-60 were required. With preirradiation using 1.0 megarep, sterility was attained with an F_0 of approximately 0.15.

INTRODUCTION

Combined radiation and heat processing of canned foods to produce commercially sterile products has been considered possibly more desirable than sterilization with either energy form alone.[1,2,3] This phase of the present project is designed to test such an idea.

It has been previously demonstrated that canned meat can be sterilized by gamma radiation alone,[1] and it is common industrial practice to sterilize foods with heat. When a study of the combined processing was considered, it was found desirable first to irradiate the inoculated packs of canned ground meat and follow this by heat processing. The sequence of irradiation followed by heat processing is based on the previously observed finding[2,3] that preirradiation sensitized the anaerobic bacterial spores to the lethal properties of heat and that the sensitization persists during subsequent storage until heat processing is effected.

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

process for further investigation. The study has been concerned with sterilizing inoculated packs prepared from previously heat-sterilized ground beef packed in No. 1 picnic tin cans before inoculation. Data have been accumulated to establish possible combined sterilization treatments under these conditions at two levels of inoculation with Clostridium botulinum 213B spores.

MATERIALS AND METHODS

a. Packing.—Lean ground beef is purchased locally from The University of Michigan food stores. The meat is placed in shallow pans and autoclaved at 15 psig steam pressure for one-half hour. Excess liquid is poured off, and the hot meat is packed into 28 No. 1 picnic tin cans, four of which have previously been fitted with O. F. Ecklund thermocouples. Covers are set loosely on the cans of meat which are then placed in an autoclave where they are sterilized at 17 psig steam pressure for one hour. Next, each can is removed individually from the hot autoclave and the meat is inoculated with 2 ml of a spore suspension. The cans are then sealed in a commercial-type closing machine, immersed in cold tap water for about 20 minutes, and finally placed in ice water for an hour. Experimental cans are then either irradiated or temporarily stored in a refrigerator, as required. The 8 controls are placed in an incubator immediately, while the experimental cans are incubated after processing. Processed cans are quickly cooled by immersion in cold water before incubation. Incubation is carried out at 37°C or 30°C, as indicated.

b. Irradiation.—The canned meat is irradiated in the "center well" of the large cobalt-60 source here at The University of Michigan. The temperature of the meat is kept below 4°C during irradiation.

c. Heat Processing.—Several false starts were made before a heat-processing technique was developed that yielded reproducible data. As finally developed, the process is now carried out as follows:

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

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

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

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

4. When the cans have equilibrated at some temperature between 170° and 180°F, the water-bath temperature is quickly brought to 230°F by introducing steam into the autoclave. Processing time to attain the desired F_0 value, less the cooling increment, is now provided.

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

6. The four experimental cans of meat are incubated. The two cans containing thermocouples are again refrigerated; they are used a second time only. Incubation was carried out at 37°C for the first series of runs, i.e., for those packs inoculated with approximately 5,000,000 C. botulinum 213B spores per can. The second series, or those cans inoculated with approximately 300 spores per can, were incubated at 29°C.

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

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

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

RESULTS

Canned ground beef packed in No. 1 picnic tin cans was sterilized

by combined irradiation—heat processing technique.

a. As shown in Figs. 1-P and 2-P and Tables I-P through IV-P, an induction level of preirradiation with gamma rays was required before the sensitization of spores became significantly important. This level was found to be approximately 1 megarep for C. botulinum 213B spores.

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

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

TABLE I-P

F_0 Value Required to Sterilize Ground Beef in No. 1 Picnic Tin Cans Previously Inoculated with Approximately 5,000,000 C. botulinum 213B Spores Per Can

Run No. - C-1

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Spores - 10,700,000 C. botulinum 213B spores per can
Incubation Temperature - 37°

F_0	Can No.	Days to Gas Formation
Inoculated control	1	2
	2	2
	3	2
	4	2

F_o	Can No.	Days to Gas Formation
Noninoculated control	1	-
	2	-
	3	-
	4	-
Can 1, 0.26	1	4.5
Can 2, 0.31	2	5
	3	5
	4	5
Can 1, 1.00	5	6.5
Can 2, 1.00	6	7
	7	6
	8	11
Can 1, 0.80	9	6.5
Can 2, 1.25	10	7.5
	11	7.5
	12	6.5
Can 1, 0.53	13	6.5
Can 2, 0.57	14	5.5
	15	7.5
	16	5.5

Conclusion: The F_o required to sterilize ground beef packed in No. 1 picnic tin cans and inoculated with 10,700,000 C. botulinum 213B spores is more than, but close to, 1.00.

Remarks: Used equilibration bath temperature of 210°F and sterilizing bath at 230°F.

Run No. - C-2

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Spores - 7,200,000 C. botulinum 213B spores per can
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Inoculated control	1	1.5
	2	1.5
	3	1.5
	4	1.5

F_o	Can No.	Days to Gas Formation
Noninoculated control	1	-
	2	-
	3	-
	4	-
Can 1, 0.28	1	3.5
Can 2, 0.39	2	3.5
	3	3.5
	4	3.5
Can 1, 0.70	5	-
Can 2, 0.73	6	-
	7	-
	8	3.5
Can 1, 0.75	9	3.5
Can 2, 1.01	10	4.5
	11	4.5
	12	-
Can 1, 1.24	13	-
Can 2, 1.29	14	-
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans and inoculated with 7,200,000 C. botulinum 213B spores per can was sterilized in the F_o range of 0.75 to 1.29.

Remarks: A preheat water-bath temperature of 210°F and sterilizing bath temperature of 230°F were used.

Run No. - C-3

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Spores - 10,700,000 C. botulinum 213B spores per can
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Inoculated control	1	1.5
	2	1.5
	3	1.5
	4	1.5

F_o	Can No.	Days to Gas Formation
Noninoculated control	1	-
	2	-
	3	-
	4	-
Can 1, 0.36	1	6
Can 2, 0.49	2	6
	3	6
	4	6
Can 1, 0.93	5	-
Can 2, 0.93	6	-
	7	-
	8	-
Can 1, 1.18	9	-
Can 2, 1.53	10	-
	11	-
	12	-
Can 1, 1.59	13	-
Can 2, 1.82	14	-
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans and inoculated with 10,700,000 C. botulinum 213B spores per can was sterilized in the F_o range of 0.36 to 0.93.

Remarks: A preheat water-bath temperature of 190°F and a sterilizing bath temperature of 230°F were used.

TABLE II-P

Gamma-Radiation Dosage Required to Sterilize Ground Beef in No. 1 Picnic Tin Cans Previously Inoculated with Approximately 5,000,000 C. botulinum 213B Spores Per Can

Run No. - CB-15

Can Size	- No. 1 Picnic (211 x 400)
Product	- Ground Beef
Inoculum	- 5,000,000 C. botulinum 213B spores per can
Incubation Temperature	- 37°C

Radiation Dosage (megarep)	Can No.	Days to Gas Formation
Noninoculated control - no irradiation	1	-
	2	-
	3	-
	4	-
Inoculated control - no irradiation	21	2
	22	2
	23	2
	24	2
3.42	1	-
	2	4
	3	4
	4	4
3.96	5	-
	6	-
	7	-
	8	-
2.52	9	4
	10	3
	11	3
	12	3
2.16	13	2
	14	3
	15	3
	16	3
2.88	17	4
	18	3
	19	5
	20	4

Conclusion: Ground beef packed in No. 1 picnic tin cans and inoculated with 5,000,000 C. botulinum 213B spores per can was sterilized with 3.42 to 3.96 megarep of gamma radiation from cobalt-60.

TABLE III-P

F_o Required to Sterilize Ground Beef Packed in No. 1 Picnic Tin Cans, Inoculated with Approximately 5,000,000 *C. botulinum* 213B Spores Per Can, and Then Irradiated with Gamma Rays from Cobalt-60 Before Heat Processing

Run No. - CB-3

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 2,300,000 *C. botulinum* 213B spores per can
Preirradiation - 1.35 megarep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Inoculated control	1	2
	2	2
	3	2
	4	2
Noninoculated control	1	-
	2	-
	3	-
	4	-
0.36	1	-
	2	-
	3	-
	4	-
1.03	5	-
	6	-
	7	-
	8	-
0.23	9	-
	10	-
	11	-
	12	-
0.63	13	-
	14	-
	15	-
	16	-

Run No. CB-4

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 5,200,000 C. botulinum 213B spores per can
Preirradiation - 1,350,000 rep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Inoculated control	1	1
	2	1
	3	1
	4	1
Noninoculated control	1	-
	2	-
	3	-
	4	-
Irradiation control	17	1.5
Without heat process-	18	1.5
ing but using	19	1.5
1,350,000 rep	20	1.5
Can 1, 0.11	1	4
Can 2, 0.11	2	-
	3	-
	4	-
Can 1, 0.21	5	-
Can 2, 0.21	6	-
	7	-
	8	-
Can 1, 0.42	9	-
Can 2, 0.42	10	-
	11	-
	12	-
Can 1, 0.62	13	-
Can 2, 0.62	14	-
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 5,200,000 C. botulinum 213B spores per can, and then irradiated with 1.35 megarep of gamma rays from cobalt-60, subsequently required an F_o between 0.11 and 0.21 for sterilization.

Run No. CB-5

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 6,300,000 C. botulinum 213B spores per can
Preirradiation - 675,000 rep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Inoculated control	1	2
	2	2
	3	2
	4	2
Noninoculated control	1	-
	2	-
	3	-
	4	-
Can 1, 0.12	1	3
Can 2, 0.12	2	3
	3	3
	4	3
Can 1, 0.36	5	5
Can 2, 0.36	6	4
	7	4
	8	4
Can 1, 0.47	9	8
Can 2, 0.47	10	-
	11	-
	12	-
Can 1, 0.58	13	-
Can 2, 0.58	14	4*
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 6,300,000 C. botulinum 213B spores per can, then irradiated with 0.675 megarep of gamma rays from cobalt-60, subsequently required an F_o greater than 0.58 for sterilization.

*Toxin present by animal inoculation test.

Run No. CB-6

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 6,000,000 C. botulinum 213B spores per can
Preirradiation - 1,000,000 rep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Inoculated control	1	2
	2	2
	3	2
	4	2
Noninoculated control	1	-
	2	-
	3	-
	4	-
Can 1, 0.24	1	5
Can 2, 0.39	2	6
	3	6
	4	7
Can 1, 0.41	5	-
Can 2, 0.59	6	7
	7	-
	8	7
Can 1, 0.80	9	-
Can 2, 0.80	10	-
	12	-
	13	-
Can 1, 0.04	11	5
Can 2, 0.09	14	4
	15	5
	16	4

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 6,000,000 C. botulinum 213B spores per can, and then irradiated with 1.00 megarep of gamma radiation from cobalt-60, subsequently required an F_o between 0.41 and 0.80 for sterilization.

Run No. CB-7

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 3,800,000 C. botulinum 213B spores per can
Preirradiation - 1,200,000 rep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Inoculated control	1	2
	2	2
Noninoculated control	1	-
	2	-
	3	-
	4	-
Can 1, 0.66	1	-
Can 2, 0.77	2	-
	3	-
	4	-
Can 1, 0.55	5	-
Can 2, 0.55	6	-
	7	-
	8	-
Can 1, 0.25	9	-
Can 2, 0.29	10	-
	11	-
	12	-
Can 1, 0.09	13	-
Can 2, 0.11	14	-
	15	-
	16	6

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 3,800,000 C. botulinum 213B spores per can, and then irradiated with 1.200 megarep of gamma radiation from cobalt-60, subsequently required an F_o between 0.09 and 0.29 for sterilization.

Run No. CB-8

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 5,000,000 C. botulinum 213B
Preirradiation - 500,000 rep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Noninoculated control	1	2
	2	
	3	
	4	
Inoculated control	1	2
	2	2
	3	2
	4	2
Can 1, 1.40	1	-
Can 2, 0.85	2	-
	3	-
	4	-
Can 1, 0.745	5	-
Can 2, 0.639	6	-
	7	-
	8	-
Can 1, 1.05	9	-
Can 2, 0.73	10	-
	11	-
	12	-
Can 1, 0.881	13	-
Can 2, 0.540	14	-
	15	-
	16	-
Nonirradiated controls	17	-
	18	-
Can 1, 0.86	19	-
	20	-
Can 1, 1.18	21	-
	22	-
	23	-
	24	-

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 5,000,000 C. botulinum 213B spores per can, and then irradiated with 0.500 megarep of gamma radiation from cobalt-60, required an F_o greater than 0.85 for sterilization.

Run No. CB-9

Can Size - No. 1 Picnic
Product - Ground Beef
Inoculum - 5,000,000 C. botulinum 213B spores per can
Preirradiation - 1,500,000 rep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Noninoculated controls	1	
	2	
	3	
Inoculated controls	1	2
	2	2
	3	2
	4	2
Can 1, 0.029	1	2
	2	2
	3	2
	4	2
Can 1, 0.10	5	3
Can 2, 0.11	6	3
	7	3
	8	-
Can 1, 0.013	9	2
Can 2, 0.019	10	2
	11	3
	12	3
Can 1, 0.062	13	5
Can 2, 0.081	14	4
	15	
	16	

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 5,000,000 C. botulinum 213B spores per can, and then irradiated with 1.500 megarep of gamma radiation from cobalt-60, required an F_o greater than 0.11 for sterilization.

Run No. CB-10

Can Size - No. 1 Picnic
Product - Ground Beef
Inoculum - 6,000,000 *C. botulinum* 213B spores per can
Preirradiation - 500,000 rep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Noninoculated controls	1	42
	2	
	3	
	4	
Inoculated controls	17	2.5
	18	2.5
	19	2.5
	20	2.5
Can 1, 0.43	1	5
Can 2, 0.26	2	6
	3	5
	4	6
Can 1, 0.56	5	6
Can 2, 0.51	6	6
	7	-
	8	6
Can 1, 1.06	9	
	10	
	11	
	12	
Can 1, 0.84	13	9
Can 2, 0.77	14	
	15	
	16	7
Nonirradiated controls	20	7
Can 1, 0.81	21	7
Can 2, 0.68	22	6
	23	7
Can 1, 0.98	24	
	25	
	26	
	27	8
	19	

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 6,000,000 C. botulinum 213B spores per can, and then irradiated with 0.500 megarep of gamma radiation from cobalt-60, required an F_0 between 0.77 and 1.06 for sterilization.

Run No. CB-11

Can Size - No. 1 Picnic
Product - Ground Beef
Inoculum - 12,000,000 C. botulinum 213B spores per can
Preirradiation - 1,500,000 rep
Incubation Temperature - 37°C

F_0	Can No.	Days to Gas Formation
Noninoculated controls	1	5*
	2	-
	3	-
	4	-
Inoculated controls	17	2
	18	2
	19	2
	20	2
Can 1, 0.329	1	5
Can 2, 0.206	2	
	3	
	4	
Can 1, 0.125	5	3
Can 2, 0.072	6	-
	7	-
	8	3
Can 1, 0.047	9	3
Can 2, 0.033	10	3
	11	3
	12	3
Can 1, 0.147	13	-
Can 2, 0.087	14	3
	15	6
	16	4

Conclusion: None.
*Toxin present by mouse inoculation test.

Run No. CB-12

Can Size - No. 1 Picnic
Product - Ground Beef
Inoculation - 5,000,000 C. botulinum 213B spores per can
Preirradiation - 1,500,000 rep
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Noninoculated controls	1	
	2	
	3	
	4	
Inoculated controls	17	2
	18	2
	19	2
	20	2
Can 1, 0.15	1	3
Can 2, 0.04	2	3
	3	3
	4	3
Can 1, 0.27	5	
Can 2, 0.26	6	
	7	
	8	
Can 1, 0.063	9	2
	10	3
	11	2
	12	3
Can 1, 0.39	13	
Can 2, 0.25	14	
	15	
	16	

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 5,000,000 C. botulinum 213B spores per can, and then irradiated with 1.500 megarep of gamma radiation from cobalt-60, subsequently required an F_o between 0.063 and 0.27 for sterilization.

TABLE IV-P

F_o Value Required to Sterilize Ground Beef Packed in No. 1 Picnic Tin Cans, Inoculated with Approximately 300 C. botulinum 213B Spores, and Then Heat Processed

Run No. CB-17

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 250 C. botulinum 213B spores per can
Incubation Temperature - 37°C

F_o	Can No.	Days to Gas Formation
Noninoculated control	1	-
	2	-
	3	-
	4	-
Inoculated control	17	3
	18	3
	19	3
	20	3
0.21	1	-
	2	-
	3	-
	4	-
0.51	5	-
	6	-
	7	-
	8	-
0.28	9	-
	10	-
	11	-
	12	-
0.37	13	-
	14	-
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 250 C. botulinum 213B spores per can, and then heat processed, requires an F_o of at least 0.21 for sterilization when incubated at 37°C.

Run No. CB-18

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 250 C. botulinum 213B spores per can
Incubation Temperature - 29°C

F_o	Can No.	Days to Gas Formation	Remarks
Noninoculated control	1	4	no toxin
	2	-	
	3	14	no toxin
	4	-	
Inoculated control	17	3	
	18	3	
	19	3	toxin
	20	3	
Can 1, 0.31	1	7	no toxin
Can 2, 0.26	2		
	3		
	4		
Can 1, 0.14	5	4	toxin
	6	4	
	7	4	no toxin
	8	4	
Can 1, 0.079	9	3	toxin
Can 2, 0.058	10	3	toxin
	11	3	
	12	3	
0.035	13	3	
	14	3	
	15	3	toxin
	16	3	

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 250 C. botulinum 213B spores per can, and then heat processed, requires an F_o between 0.14 and 0.31 for sterilization.

Run No. CB-19

Can Size - No. 1 Picnic (211 x 400)
Product - Canned Beef
Inoculum - 400 C. botulinum 213B spores per can
Incubation Temperature - 29°C

F_o	Can No.	Days to Gas Formation	Remarks
Inoculated control	19	4	
	20	1	
	21	4	toxin
	22	4	
Noninoculated control	1	17	no toxin
	2	-	
Can 1, 0.03	1	6	
Can 2, 0.045	2	6	
	3	4	
	4	6	
F_o = 0.084	5	5	
	6	4	toxin
	7	4	toxin
	8	5	
F_o = 0.15	9	-	
	10	-	
	11	-	
	12	8	toxin
Can 1, 0.26	13	-	
0.45	14	-	
	15	-	
	16	4	no toxin

Remark: An additional experiment on autoclaving carried out as part of this run established the fact that the cans were not being adequately processed by our preliminary autoclaving treatment. This explained the positive controls obtained in this and, occasionally, in previous runs. After considerable effort, it was found that the automatic exhausting system was almost completely plugged with meat particles. So the cans were being processed in an incompletely exhausted autoclave. This was immediately corrected.

Conclusion: Based on toxin tests in mice in addition to the observation of gas production, this run shows that ground beef packed in No. 1 picnic tin cans, inoculated with 400 C. botulinum 213B spores per can, requires an F_o between 0.15 and 0.45 for sterilization.

Run No. CB-24

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 300 C. botulinum 213B spores per can
Heat Processed Only -
Incubation Temperature - 29°C

F_o	Can No.	Days to Gas Formation
Noninoculated controls	1	-
	2	-
	3	-
Inoculated control	17	6
	18	6
Can 1, 0.26	1	-
Can 2, 0.26	2	5
	3	5
	4	7
Can 1, 0.36	5	-
Can 2, 0.54	6	-
	7	7
	8	-
Can 1, 0.33	9	-
Can 2, 0.33	10	7
	11	-
	12	-
Can 1, 0.47	13	-
	14	-
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 300 C. botulinum 213B spores per can, was sterilized by heat processing to an F_o value between 0.33 and 0.47.

TABLE V-P

Gamma-Radiation Dosage Required to Sterilize Ground Beef in No. 1 Picnic Tin Cans Previously Inoculated with Approximately 300 C. botulinum 213B Spores Per Can

Run No. CB-14

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 250 C. botulinum 213B spores per can
Irradiation Only -
Incubation Temperature - 37°C

Radiation Dosage (megarep)	Can No.	Days to Gas Formation
Noninoculated control	1	33
	2	41
	3	-
	4	-
Inoculated controls	17	2.5
	18	2.5
	19	2.5
	20	2.5
1.117	33	3
	34	4
	35	3
	36	3
	37	4
	38	3
	39	4
	40	
2.300	29	-
	30	-
	31	-
	32	-
2.900	25	-
	26	-
	27	-
	28	-
3.400	17	-
	18	-
	19	-
	20	-

Radiation Dosage (megarep)	Can No.	Days to Gas Formation
4.000	21	-
	22	-
	23	-
	24	-

Remarks: 1. The indication of few bacterial spores not having been killed by the presterilization treatment does not invalidate this run.

2. The incubation temperature was 37°C instead of the 29°C used on all other runs with low spore concentration.

Conclusion: Ground beef packed in No. 1 picnic tin cans and inoculated with 250 C. botulinum 213B spores per can was sterilized with between 1.117 and 2.300 megarep of gamma radiation from cobalt-60.

Run No. CB-21

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 300 C. botulinum 213B spores per can
Irradiation Only -
Incubation Temperature - 29°C

Radiation Dosage (megarep)	Can No.	Days to Gas Formation
Noninoculated control - no irradiation	1	-
	2	-
	3	-
1.02	1	6
	2	6
	3	6
	4	6
1.36	5	7
	6	6
	7	7
	8	6
1.80	8	-
	9	-
	11	-
	12	-

Radiation Dosage (megarep)	Can No.	Days to Gas Formation
2.10	13	-
	14	-
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans and inoculated with 300 C. botulinum 213B spores per can was sterilized with 1.36 to 1.80 megarep of gamma radiation from cobalt-60.

Run No. CB-27

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 300 C. botulinum 213B spores per can
Incubation Temperature - 29°C

Radiation Dosage (megarep)	Can No.	Days to Gas Formation
Noninoculated control - no irradiation	1	-
	2	-
	3	-
	17	-
Inoculated control - no irradiation	18	6
	19	6
	20	6
1.10	5	6
	7	6
	14	6
	16	6
1.64	11	8
	1	-
	12	6
	3	-
1.92	4	-
	6	-
	13	-
	15	-

Conclusion: Ground beef packed in No. 1 picnic tin cans and inoculated with 300 C. botulinum 213B spores per can was sterilized with 1.64 to 1.92 megarep of gamma radiation from cobalt-60.

TABLE VI-P

F_o Required to Sterilize Ground Beef Packed in No. 1 Picnic Tin Cans, Inoculated with Approximately 300 C. botulinum 213B Spores Per Can, and Then Irradiated with Gamma Rays From Cobalt-60 Before Heat Processing

Run No. CB-20

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 300 C. botulinum 213B spores per can
Preirradiation - 0.500 megarep
Incubation Temperature - 29°C

F_o	Can No.	Days to Gas Formation	Remarks
Noninoculated controls	1	-	
	2	-	
	3	-	
	4	-	
Inoculated controls	17	5	
	18	5	
	19	5	toxin
	20	5	
Can 1, 0.12	1	7	
Can 2, 0.17	2	7	
Can 3, 0.15	3	7	
	4	8	
Can 1, 0.095	5	7	
Can 2, 0.085	6	7	
Can 3, 0.063	7	7	
	8	-	
Can 1, 0.022	9	-	
Can 2, 0.025	10	6	
Can 3, 0.028	11	10	
	12	6	
Can 1, 0.32	13	5	toxin
Can 2, 0.40	14	10	
Can 3, 0.38	15	-	
	16	-	

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 300 C. botulinum 213B spores per can, and then irradiated with 0.500 megarep of gamma radiation from cobalt-60, subsequently required an F_o greater than 0.40 for sterilization.

Run No. CB-22

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 300 C. botulinum 213B spores per can
Preirradiation - 0.500 megarep
Incubation Temperature - 29°C

F_o	Can No.	Days to Gas Formation
Noninoculated controls	1	-
	2	-
	3	-
	4	-
Inoculated controls	17	5
	18	5
	19	5
	20	6
0.48	1	8
	2	-
	3	12
	4	10
0.41	5	-
	6	-
	7	11
	8	-
0.29	9	-
	10	-
	11	-
	12	-
Can 1, 0.15	13	-
Can 2, 0.095	14	-
	15	10
	16	10

Conclusion: None.

Run No. CB-25

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 300 C. botulinum 213B spores per can
Preirradiation - 500,000 rep
Incubation Temperature - 29°C

F_o	Can No.	Days to Gas Formation
Noninoculated controls	1	-
	2	-
	3	-
	4	-
Inoculated controls	1	3
	2	3
Can 1, 0.31	1	6
Can 2, 0.31	2	-
	3	-
	4	-
Can 1, 0.326	5	-
Can 2, 0.481	6	-
Can 3, 0.510	7	-
	8	-
Can 1, 0.60	9	-
	10	-
	11	-
	12	-
Can 1, 0.46	13	-
Can 2, 1.28	14	-
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 300 C. botulinum 213B spores per can, and then irradiated with 0.500 megarep of gamma radiation from cobalt-60, subsequently required an F_o between 0.31 and 0.51 for sterilization.

Run No. CB-26

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 300 C. botulinum 213B spores per can
Preirradiation - 0.750 megarep
Incubation Temperature - 29°C

F_o	Can No.	Days to Gas Formation
Noninoculated controls	1	-
	2	-
	3	-
	4	-
Inoculated controls	17	4
	18	4
	19	4
Can 1, 0.34	1	-
Can 2, 0.34	2	-
	3	-
	4	-
Can 1, 0.22	5	-
Can 2, 0.22	6	-
	7	-
	8	8
Can 1, 0.12	9	8
Can 2, 0.12	10	-
	11	-
	12	-
Can 1, 0.057	13	-
Can 2, 0.065	14	-
	15	-
	16	6

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 300 C. botulinum 213B spores per can, and then irradiated with 0.750 megarep of gamma radiation from cobalt-60, subsequently required an F_o between 0.22 and 0.34 for sterilization.

Run No. CB-23

Can Size - No. 1 Picnic (211 x 400)
Product - Ground Beef
Inoculum - 300 C. botulinum 213B spores per can
Preirradiation - 1.000 megarep
Incubation Temperature - 29°C

F_o	Can No.	Days to Gas Formation
Noninoculated control	1	-
	2	-
	3	-
	4	-
Inoculated control	17	5
	18	5
	19	5
	20	6
Can 1, 0.18	1	-
Can 2, 0.16	2	-
	3	-
	4	-
Can 1, 0.25	5	-
Can 2, 0.16	6	-
	7	-
	8	-
Can 1, 0.35	9	-
Can 2, 0.30	10	-
	11	-
	12	-
Can 1, 0.086	13	8
	14	-
	15	-
	16	-

Conclusion: Ground beef packed in No. 1 picnic tin cans, inoculated with 300 C. botulinum 213B spores per can, and then irradiated with 1.000 megarep of gamma radiation from cobalt-60, was subsequently sterilized by heat processing with an F_o between 0.086 and 0.18.

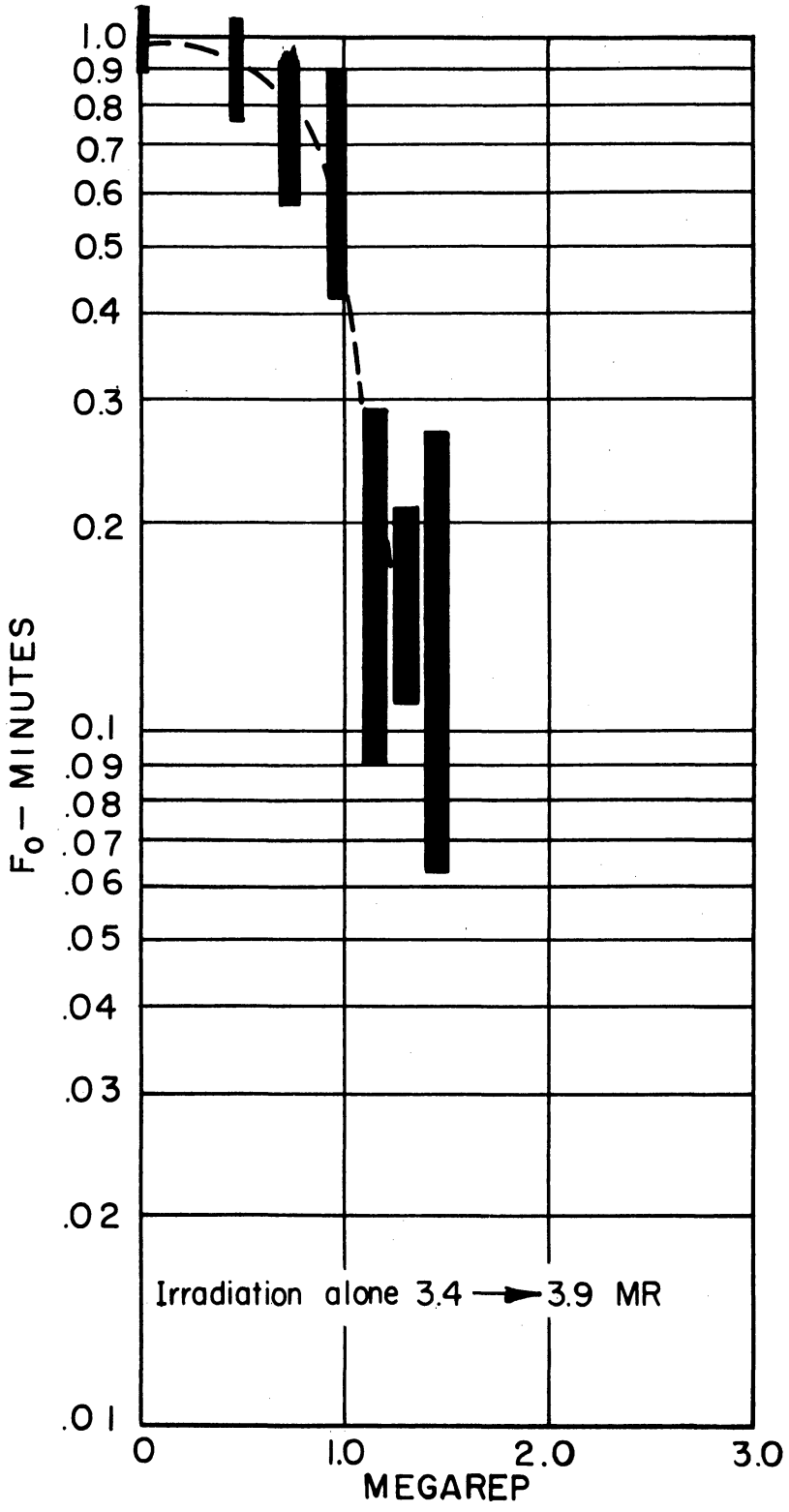

Fig. 1-P. F_o required to sterilize ground beef packed in No. 1 picnic tin cans, inoculated with approximately 5,000,000 C. botulinum 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat processing.

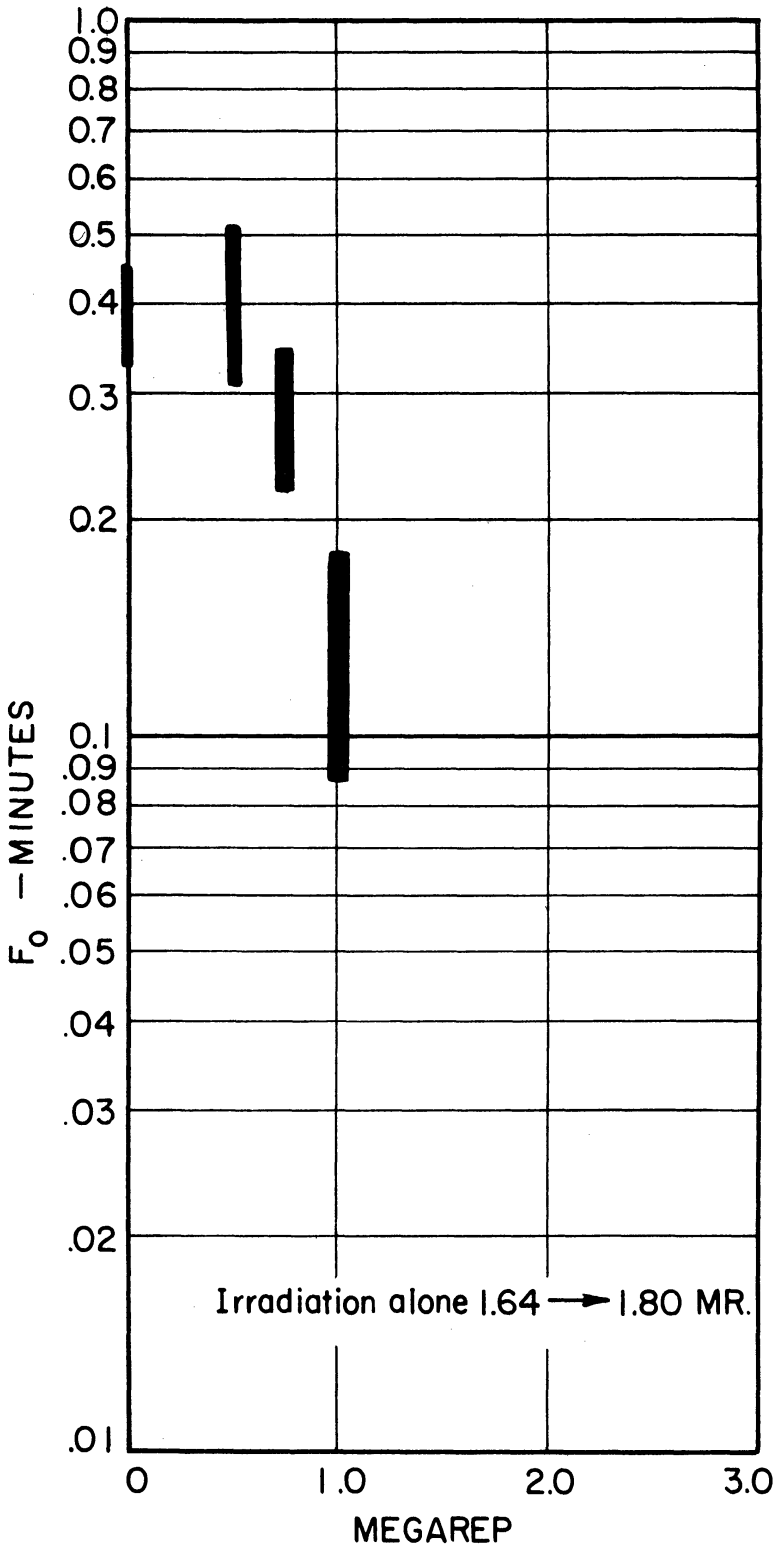

Fig. 2-P. F_0 required to sterilize ground beef packed in No. 1 picnic tin cans inoculated with approximately 300 *C. botulinum* 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat processing.

DISCUSSION

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

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

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

It will be noted from the results that the spore concentration difference between 300 and 5,000,000 spores per can did not cause a great deal of difference in the combined processing treatment required. On the other hand, where either of these treatments was used alone, spore concentration was an extremely significant factor.[1,4]

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PHASE II

EFFECT OF CHEMICAL COMPOSITION OF THE MEDIUM DURING IRRADIATION ON THE SURVIVAL OF BACTERIAL SPORES

SUMMARY

The presence of reducing agents in the chemical environment of anaerobic bacterial spores during irradiation lowers the lethal effectiveness of ionizing radiations from cobalt-60. Conversely, the presence of oxygen enhances the lethal effects of such radiations. This acquires importance when evaluating chemical additives suggested for reducing adverse flavor production by ionizing radiations since most of the chemical additives so far proposed are chemical reducing agents.

For example, in the presence of sodium hydrosulfite there was only one log cycle reduction in the number of C. botulinum 213B spores produced by one megarep of gamma radiation, whereas in phosphate buffer alone a reduction of approximately six log cycles was found with a similar amount of radiation.

Experiments with mercury compounds indicate that the probable sites of the lethal actions of ionizing radiations within anaerobic bacterial spores are associated with the presence of sulfhydryl groups at such sites. This could be the basic reason why chemical reducing agents protect these bacterial spores and oxygen acts in the opposite manner when such spores are irradiated.

INTRODUCTION

It has been shown[2] that the most important biological effects of ionizing radiations result from their ability to generate oxidizing substances such as free radicals and hydrogen peroxide, which are assumed to be effective over several molecular diameters. It has also been shown that such reactions are strongly enhanced by the presence of oxygen and that the presence of easily oxidizable materials during irradiation counteracts the oxidative effects.

Our previous work[1] has shown that these observations can be extended to aid in an understanding of the lethal actions of gamma radiations on the spores of anaerobic bacteria that are important in food preservation. The present studies have been limited to phosphate buffer or similar solutions prepared from materials of known composition as suspending media for C. botulinum or PA 3679 spores during irradiation. The work has been

directed toward learning whether the chemical additives suggested for minimizing the adverse organoleptic properties of ionizing radiations also protect these spores during irradiation. The possibility that such might be the case is obvious since both effects are considered to be at least partly due to oxidative reactions.

The results of this work will be presented under these headings, viz.:

a. Effect of reducing agents.

b. Effect of a mercury compound.

c. Effect of oxygen.

MATERIALS AND METHODS

a. Organisms.—C. botulinum 213B spores were grown for three weeks in a casitone medium at 30°C, washed in sterile water to remove the mother liquor and vegetative cells, and then collected by centrifugation. The spores were then heated at 85°C for 15 minutes to kill any remaining vegetative cells. Immediately before use, the spores were shaken with sterile glass beads for 3 minutes to disperse clumps. These stock suspensions contained approximately 4×10^8 spores per ml. For use, appropriate dilutions were made to provide more than one million spores per ml.

PA 3679 spores were grown for three weeks in pork extract broth at 30°C. They were then harvested and pretreated in a manner identical to that previously described for C. botulinum 213B spores.

b. Chemicals.—All solutions were prepared in 0.02-M concentration. Appropriate amounts of the chemicals were weighed out aseptically into sterile weighing bottles, mixed with the required solution, and used in experiments without sterilization. The following are described as examples:

Glutathione.—0.312 gm of glutathione was added to 50 ml of sterile M/15 phosphate buffer of pH 7.0.

Sodium Hydrosulfite.—0.174 gm of sodium hydrosulfite was added to 50 ml of sterile M/15 buffer of pH 7.0.

Methionine.—0.298 gm of methionine was added to 100 ml of sterile phosphate buffer of pH 7.0.

c. Control.—In every case, sterile M/15 phosphate buffer of pH 7.0 was used as the control solution.

d. Irradiation Procedures.—All samples were irradiated in flame-sealed glass vials that contained approximately 4 ml of solution. These were placed in an especially designed container which provided temperature control at 4°C. The containers and vials were then irradiated for proper intervals in the center well of the large cobalt-60 gamma radiation source.

RESULTS

a. Effect of Reducing Agents.—The data taken with C. botulinum 213B spores substantiate previous data[1] taken with C. botulinum 62A spores by showing that several chemicals reduce the lethality of gamma radiation for C. botulinum spores. Methionine, glutathione, and sodium hydrosulfite all have marked effects in this respect, as is shown in Tables I-C and II-C and in the companion Figs. 1-C and 2-C.

TABLE I-C

Effect of Methionine in M/15 Phosphate Buffer at pH 7.0 on the Lethality of Gamma Radiation from Cobalt-60 for the Spores of C. botulinum 213B

Radiation Dose Rep	Control		Methionine	
	Spores per ml	Log % Survivors	Spores per ml	Log % Survivors
0	8,700,000	2.000	7,800,000	2.000
360,000	3,150,000	1.559	5,350,000	1.836
540,000			2,260,000	1.462
720,000	83,000	-0.020	750,000	0.983
900,000	4,500	-1.287	190,000	0.387
1,080,000	128	-2.831	27,100	-0.459

TABLE II-C

Effects of Glutathione and Sodium Hydrosulfite Added to M/15 Phosphate Buffer at pH 7.0 on the Lethality of Gamma Radiation from Cobalt-60 for the Spores of C. botulinum 213B

Radiation Dose Rep	Control		Glutathione		Sodium Hydrosulfite	
	Spores per ml	Log % Survivors	Spores per ml	Log % Survivors	Spores per ml	Log % Survivors
0	7,770,000	2.000	7,500,000	2.000	7,300,000	2.000
360,000	3,600,000	1.670	5,200,000	1.841	7,100,000	1.988
540,000	645,000	0.923	3,700,000	1.693	5,700,000	1.893
720,000	57,000	-0.131	2,370,000	1.500	3,850,000	1.722
900,000	54,000	-1.154	1,100,000	1.166	2,480,000	1.531
1,080,000	49	-3.192	410,000	0.738	1,119,500	1.214
1,260,000	6	-4.108	112,500	0.176	615,000	0.926
1,440,000	0		49,500	-0.180	241,000	0.519

Data presented in Table III-C and Fig. 3-C show that glutathione and sodium hydrosulfite protect PA 3679 spores against the lethal action of gamma radiation to essentially the same degree as was shown for C. botulinum 213B spores.

The effect of pH of the suspending medium during irradiation on the lethality of gamma radiation for C. botulinum 213B spores was also studied over the range of 3.23 to 8.4. 0.1N acetate buffer was used in the pH range of 3.23 to 5.0, while an M/15 phosphate buffer was used from 6.1 to 8.4. No significant difference was noted in the percent of C. botulinum 213B spores surviving irradiation at any pH in the range of 3.23 to 8.4. This is shown in Table IV-C. There appears to be some effect on the number of spores, since there were roughly 100 times as many survivors following 0.9-megarep irradiation in the first series of experiments as were found in the second series. However, since each series of runs agrees within itself, the basic conclusion is not affected by this disparity.

b. Effect of a Mercury Compound.—It is well known that organic compounds can be protected during chemical manipulation by the preparatory formation of compounds whose nature permits regeneration of the original compound at a later time by suitable chemical or physical operations. Since the sulfhydryl groups are suspected of being involved in the sensitivity of anaerobic bacterial spores to the lethal action of gamma radiation, and since there is microbiological precedent for reversing the

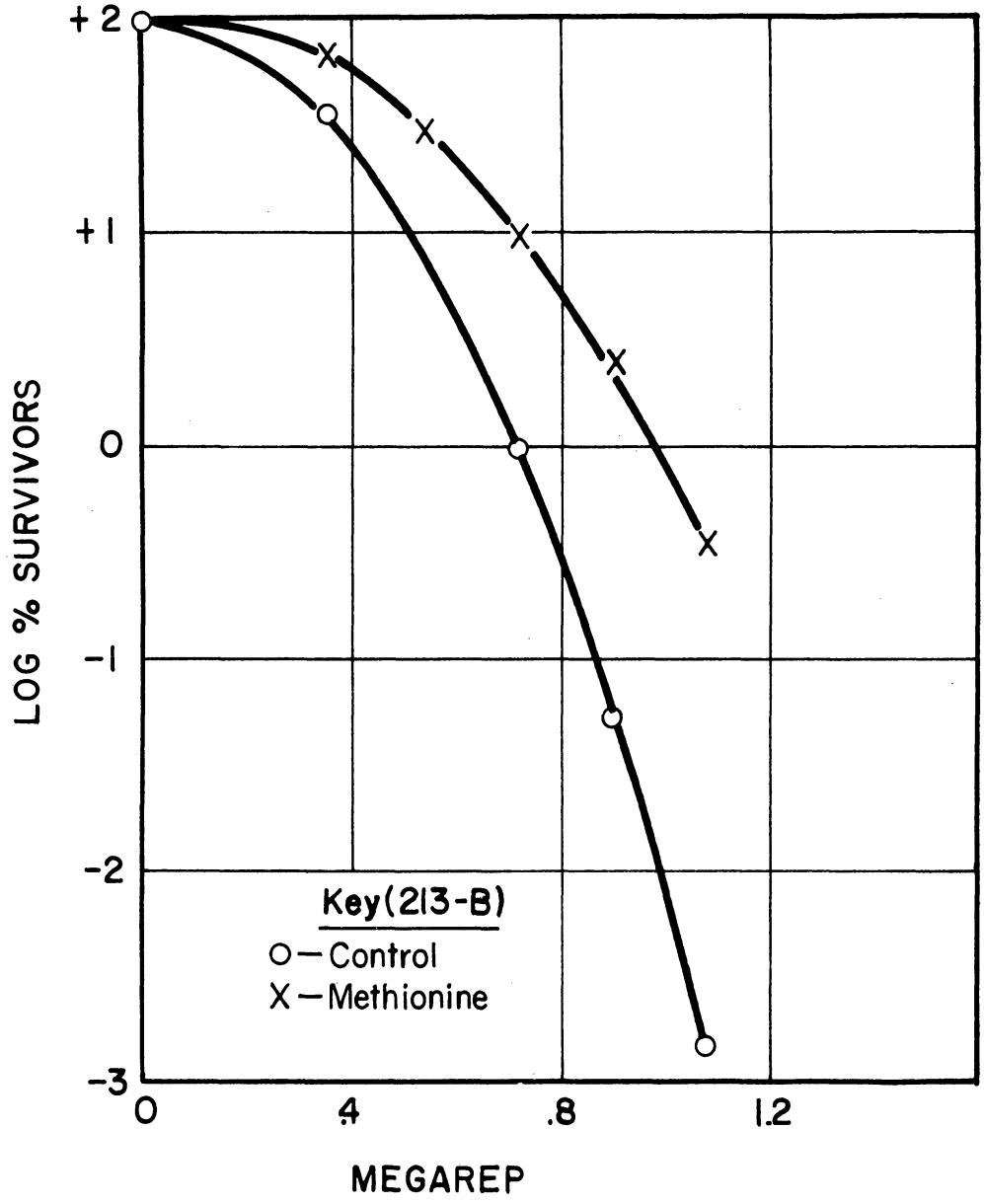

Fig. 1-C. Effect of methionine in M/15 phosphate buffer of pH 7.0 on the lethality of gamma radiation from cobalt-60 for the spores of C. botulinum 213B.

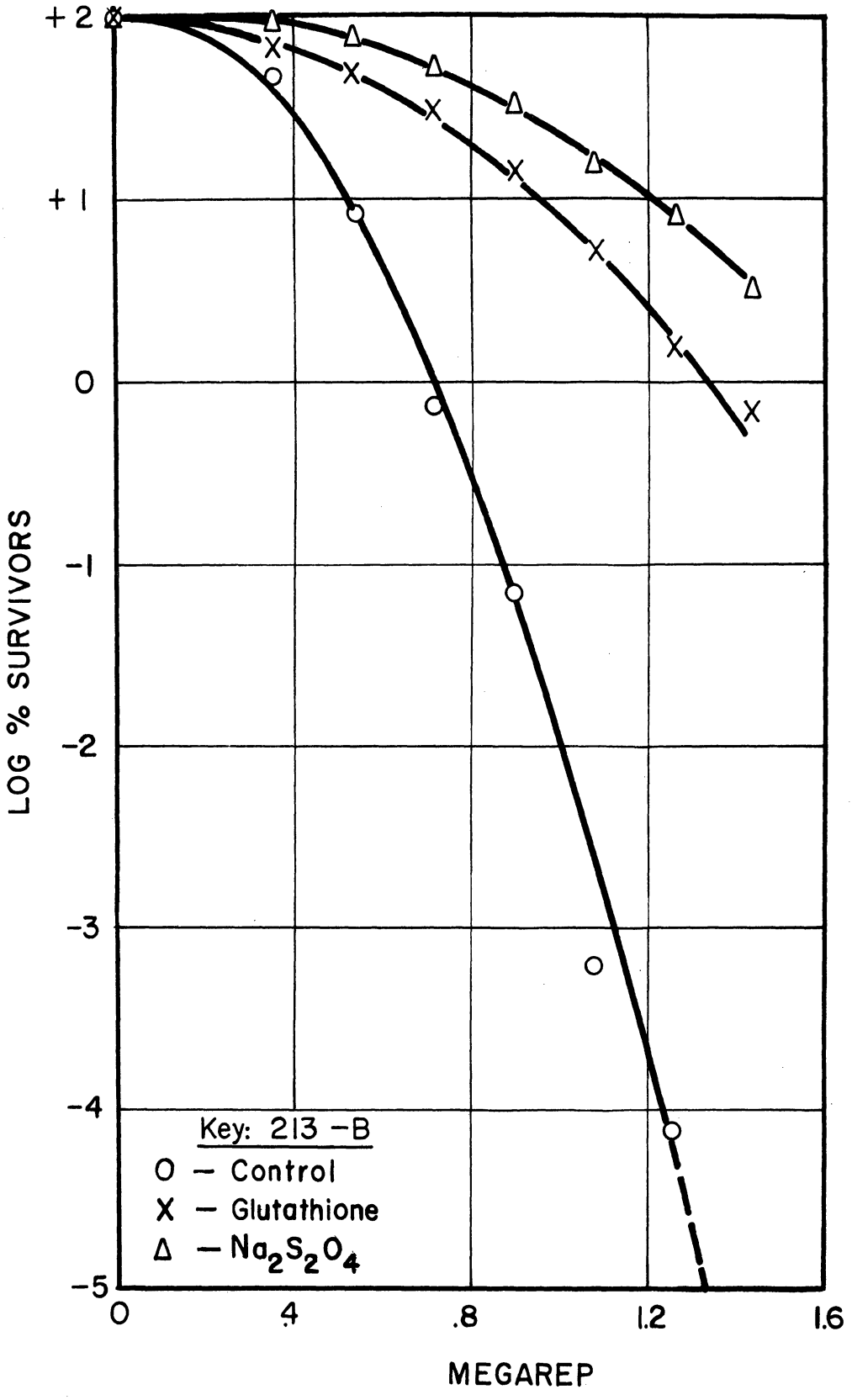

Fig. 2-C. Effects of glutathione and sodium hydrosulfite added to M/15 phosphate buffer at pH 7.0 on the lethality of gamma radiation from cobalt-60 for the spores of C. botulinum 213B.

TABLE III-C

Effect of Protective Chemicals Added to the M/15 Phosphate Buffer Used as a Suspending Medium for PA 3679 Spores During Irradiation with Gamma Rays from Cobalt-60

Rep	Spores per ml	Percent Survivors	Log Percent Survivors
a) Control - M/15 Phosphate Buffer at pH 7.0			
0	3,130,000	100	2.000
360,000	1,120,000	35.78	1.554
720,000	16,200	0.5176	-0.286
900,000	1,210	0.0387	-1.412
1,080,000	129.5	0.00414	-2.383
1,260,000	1.5	0.0000479	-4.320
1,440,000	0.5	0.000016	-4.796
b) 0.02 M Glutathione Solution in M/15 Phosphate Buffer at pH 7.0			
0	3,600,000	100	2.000
360,000	1,925,000	53.47	1.728
720,000	720,000	20.00	1.301
900,000	260,000	7.22	0.859
1,080,000	128,500	3.57	0.553
1,260,000	36,500	1.014	0.006
1,440,000	6,250	0.1736	-0.760
c) 0.02 M Sodium Hydrosulfite Solution in M/15 Phosphate Buffer at pH 7.0			
0	3,570,000	100	2.000
360,000	2,700,000	75.63	1.879
720,000	1,700,000	47.62	1.678
900,000	750,000	21.01	1.322
1,080,000	440,000	12.33	1.091
1,260,000	195,000	5.462	0.737
1,400,000	88,000	2.465	0.392

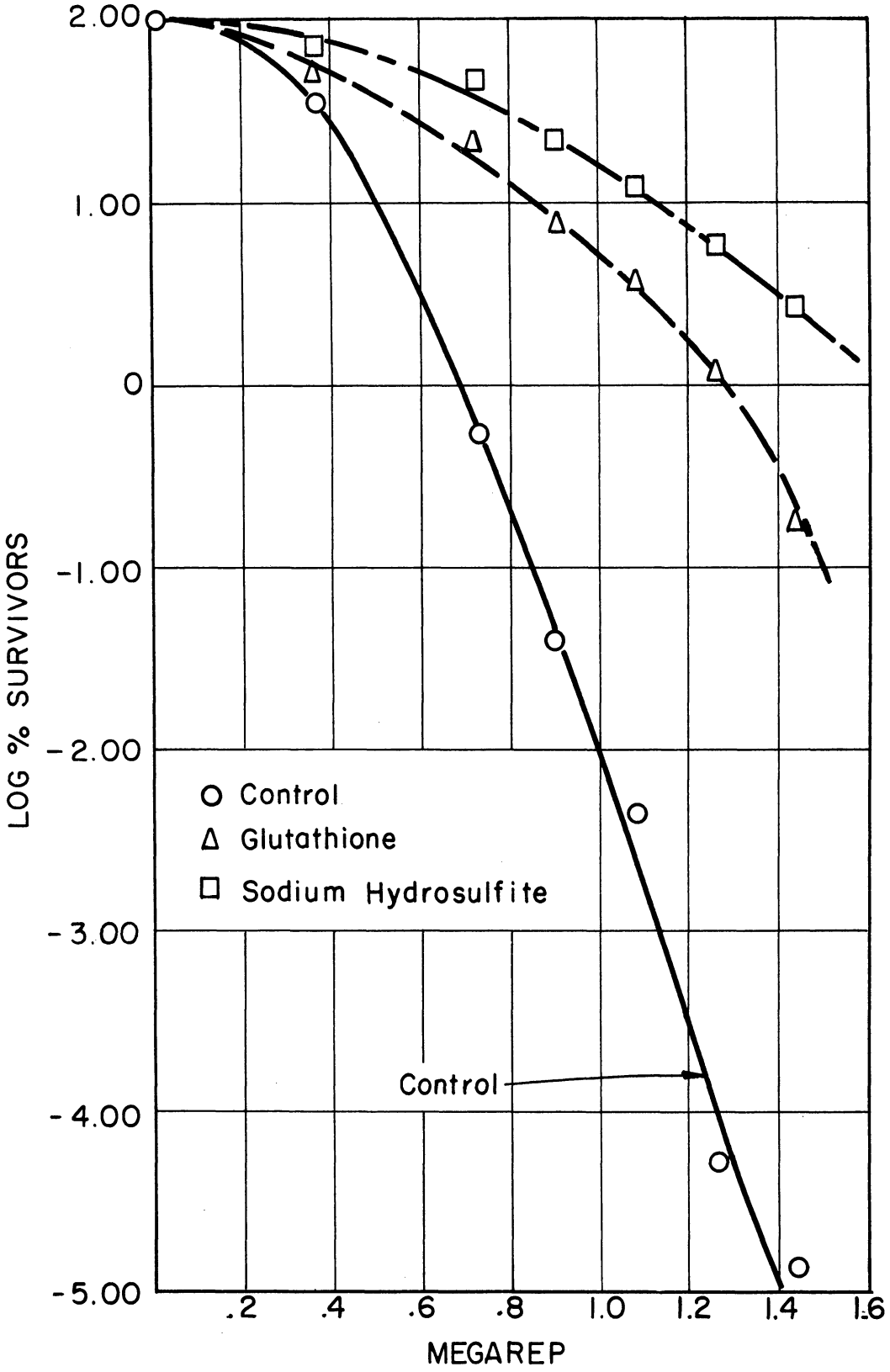

Fig. 3-C. Effect of protective chemicals at 0.02 M concentration when present in the M/15 phosphate buffer (pH 7.0) used as a suspending medium for PA 3679 spores during irradiation with gamma rays from cobalt-60.

TABLE IV-C

Effect of the pH of the Medium in Which C. botulinum 213B Spores Were Suspended During Irradiation on the Lethality of Gamma Rays from Cobalt-60 for These Spores

Rep	Spores per ml	Percent Survivors	Log Percent Survivors
pH 3.4			
0	10,900,000	100	2.000
360,000	8,400,000	77.06	1.887
720,000	1,910,000	17.52	1.244
900,000	725,000	6.651	0.823
1,080,000	195,500	1.794	0.254
pH 6.75			
0	13,000,000	100	2.000
360,000	8,650,000	66.54	1.823
720,000	5,300,000	40.77	1.610
900,000	2,050,000	15.77	1.198
1,080,000	945,000	7.27	0.862
pH 7.01			
0	10,800,000	100	2.000
360,000	8,200,000	75.93	1.880
720,000	3,065,000	28.38	1.453
900,000	1,940,000	17.96	1.254
1,080,000	665,000	6.157	0.789
pH 8.40			
0	6,000,000	100	2.000
360,000	2,230,000	37.17	1.570
540,000	330,000	5.50	0.740
720,000	42,000	0.70	-0.155
900,000	3,100	0.0517	-1.287
pH 7.17			
0	5,800,000	100	2.000
360,000	2,155,000	37.16	1.570
540,000	240,000	4.138	0.617
720,000	69,500	1.200	0.079
900,000	5,650	0.0974	-1.011

TABLE IV-C (Concl.)

Rep	Spores per ml	Percent Survivors	Log Percent Survivors
pH 6.10			
0	4,870,000	100	2.000
360,000	1,910,000	39.32	1.594
540,000	255,000	5.236	0.719
720,000	56,000	1.150	0.061
900,000	3,740	0.0768	-1.115
pH 5.00			
0	4,730,000	100	2.000
360,000	1,180,000	24.95	1.397
540,000	174,000	3.679	0.566
720,000	20,400	0.4313	-0.365
900,000	1,625	0.0344	-1.463
pH 4.00			
0	3,900,000	100	2.000
360,000	845,000	21.67	1.336
540,000	139,500	3.577	0.554
720,000	8,650	0.2218	-0.654
900,000	176	0.04513	-1.346
pH 3.23			
0	1,553,000	100	2.000
360,000	420,000	27.04	1.432
540,000	75,000	4.829	0.684
720,000	5,500	0.354	-0.451
900,000	850	0.05473	-1.262

"bactericidal" effects of mercury compounds, it appeared reasonable that such compounds might be useful for learning more about the mechanism of radiation damage to such spores.

For this purpose C. botulinum 213B spores, prepared as previously described, were suspended in solutions of p-chloromercuribenzoate. Subsequent to irradiation, the spores were counted in the usual pork extract media to which sufficient sodium thioglycollate had been added to insure reversal of mercury combinations with the spores. Incubation was carried out at 30°C.

Table V-C and Fig. 4-C present initial evidence indicating that some protection is offered to C. botulinum spores by p-chloromercuribenzoate

and that this protection varies directly with the concentration of this compound in the suspending medium.

TABLE V-C

The Effect of Different Molar Concentrations of p-Chloromercuribenzoate on the Lethality of Gamma Rays from Cobalt-60 on the Spores of C. botulinum 213B

Concentration of Hg cpd Present	Megarep	Number of Spores	Log % Survivors
0.02 M	0.000	4,930,000	2.000
	0.775	1,950	-1.403
	1.085	15	-3.517
	1.240	0.5	-4.994
0.05 M	0.000	6,700,000	2.000
	0.775	12,000	-0.747
	1.085	93	-2.858
	1.240	4	-4.282
0.08 M	0.000	7,600,000	2.000
	0.775	18,550	-0.612
	1.085	170	-2.652
	1.240	7	-4.036
0.10 M	0.000	6,800,000	2.000
	0.775	24,950	-0.435
	1.085	305	-2.348
	1.240	23	-3.480

c. Effect of Oxygen.--Since oxygen is known to enhance the damaging effects of ionizing radiation,[2,3] the influence of oxygen on the effects of sodium hydrosulfite and of glutathione present in the suspending media was studied. For this purpose C. botulinum 213B spores were suspended in (1) phosphate buffer, (2) phosphate buffer containing 0.02 M glutathione, and (3) phosphate buffer containing 0.02 M sodium hydrosulfite. The phosphate buffer was M/15 and had a pH of 7.0. Each batch of tubes containing spore suspension was treated in one of the following ways:

1. The tubes were sealed in an atmosphere of air.

2. Pure nitrogen was bubbled through this liquid, and then the tubes were sealed in an atmosphere of nitrogen.

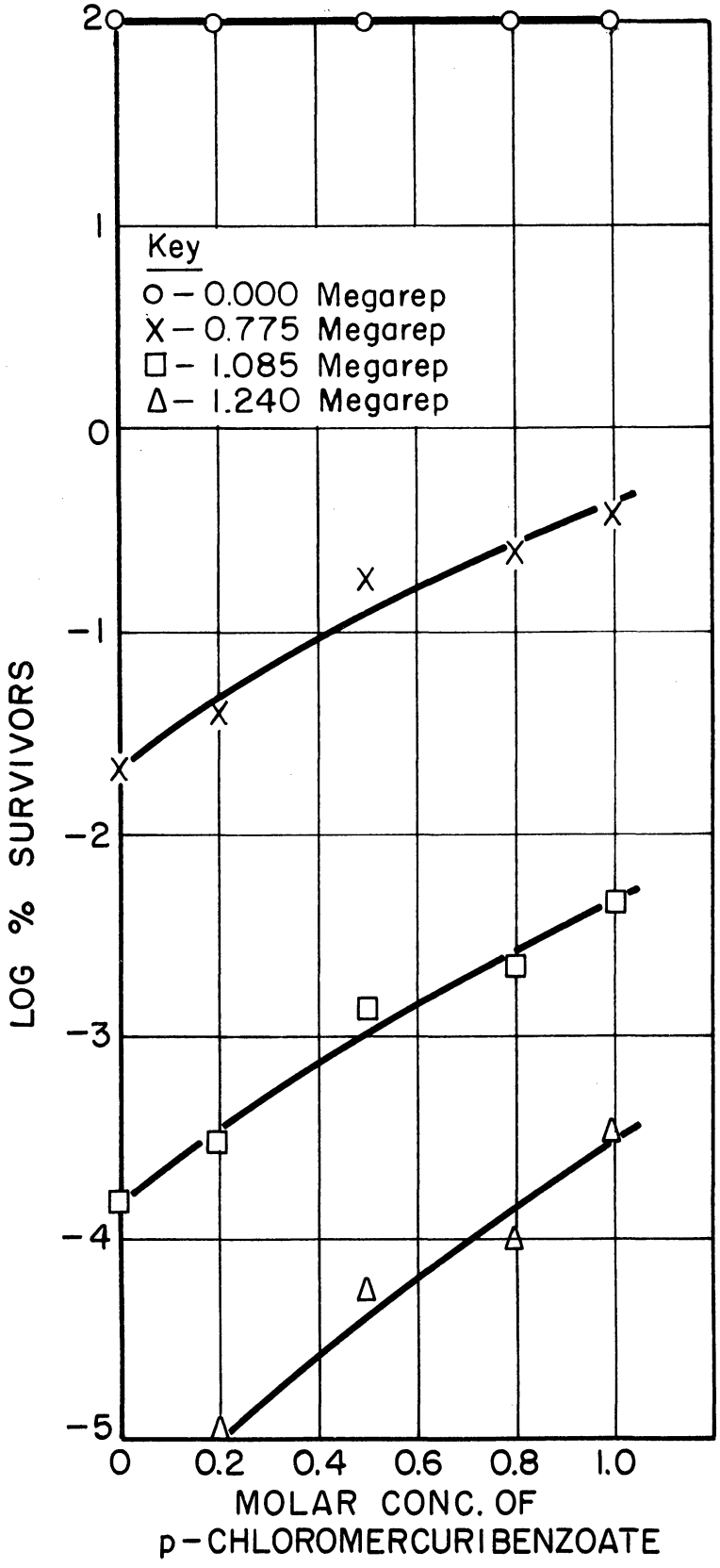

Fig. 4-C. The effect of different molar concentrations of p-chloromercuribenzoate on the lethality of gamma rays from cobalt-60 on the spores of C. botulinum 213B.

3. Pure oxygen was bubbled through the medium, and the tubes were then sealed in an atmosphere of oxygen.

The data in Table VI-C and Figs. 5-C through 7-C show the effects of varying oxygen tension during irradiation on the lethal action of gamma rays from cobalt-60 for the spores of C. botulinum 213B when different suspending media were used. Figure 5-C shows such data for a suspending medium composed of M/15 phosphate buffer at pH 7.0, Fig. 6-C for glutathione, and Fig. 7-C for sodium hydrosulfite.

It will be noted that the protective effect of sodium hydrosulfite is reduced in an atmosphere of pure oxygen but that there is little difference between an air and a nitrogen atmosphere in this respect. On the other hand, glutathione shows considerably reduced protective ability in an air atmosphere as compared with a nitrogen atmosphere, and there is even further reduction of the protective effect of glutathione when pure oxygen is substituted for air.

From all of these experiments, we conclude that reduced sulfhydryl groups present in the spores protect the spores against the lethal action of gamma rays from cobalt-60. On the other hand oxidized sulfhydryl groups do not provide such protection.

TABLE VI-C

Effect of Oxygen Tension on the Protection Afforded C. botulinum 213B Spores by Sodium Hydrosulfite and Glutathione Against the Lethal Effects of Gamma Rays of Cobalt-60

Gaseous Atmosphere	Megarep	Number of Organisms	Log % Survivors
Phosphate Buffer			
Air	0.000	5,730,000	2.000
	0.465	1,310,000	1.359
	0.775	31,500	-0.260
	0.930	675	-1.929
	1.085	190	-2.479
Oxygen	0.000	6,530,000	2.000
	0.465	485,000	0.871
	0.775	1,500	-1.639
	0.930	27	-3.384
	1.085	1	-4.815

TABLE VI-C (Cont.)

Gaseous Atmosphere	Megarep	Number of Organisms	Log % Survivors
Nitrogen	0.000	5,270,000	2.000
	0.465	1,810,000	1.536
	0.775	144,000	0.437
	0.930	20,700	-0.406
	1.085	3,000	-1.245
Glutathione			
Air	0.000	6,630,000	2.000
	0.482	1,350,000	1.309
	0.775	420,000	0.802
	0.930	208,500	0.498
	1.085	37,500	-0.247
Oxygen	0.000	7,750,000	2.000
	0.482	140,000	0.267
	0.775	2,650	-1.456
	0.930	255	-2.473
	1.085	21	-3.557
Nitrogen	0.000	6,100,000	2.000
	0.465	2,350,000	1.586
	0.775	985,000	1.208
	0.930	710,000	1.066
	1.085	250,000	0.613
Sodium Hydrosulfite			
Air	0.000	5,400,000	2.000
	0.465	5,300,000	1.992
	0.775	2,465,000	1.659
	0.930	1,510,000	1.447
	1.085	780,000	1.160
Oxygen	0.000	3,830,000	2.000
	0.465	3,150,000	1.915
	0.775	1,140,000	1.474
	0.930	131,000	0.534
	1.085	90,000	0.371

TABLE VI-C (Concl.)

Gaseous Atmosphere	Megarep	Number of Organisms	Log % Survivors
Nitrogen	0.000	7,300,000	2.000
	0.465	3,850,000	1.722
	0.775	3,100,000	1.628
	0.930	2,350,000	1.508
	1.085	1,350,000	1.267

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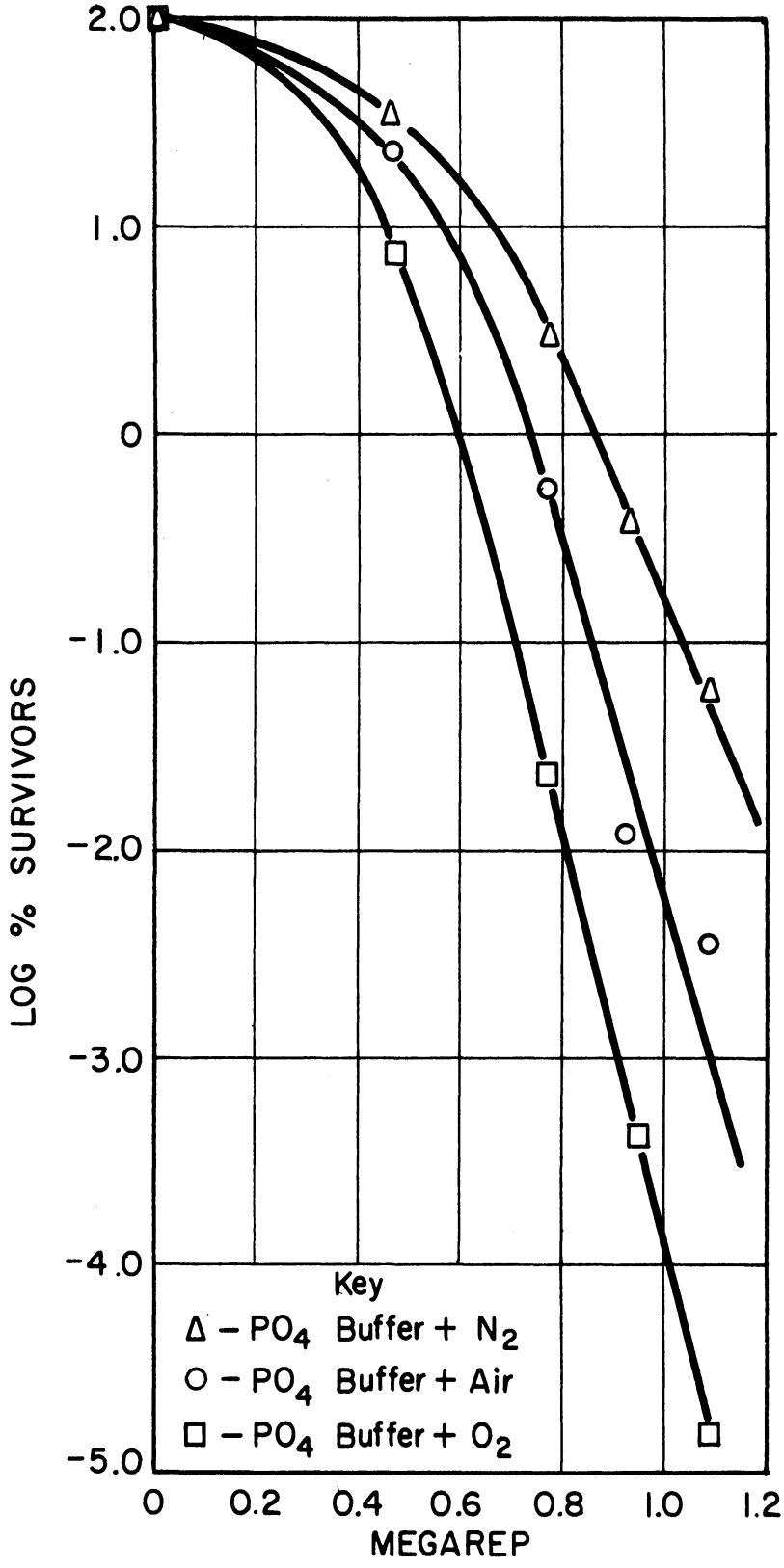

Fig. 5-C. The effect of oxygen tension on the lethality of gamma rays from cobalt-60 on the spores of C. botulinum 213B suspended in M/15 phosphate buffer at pH 7.0.

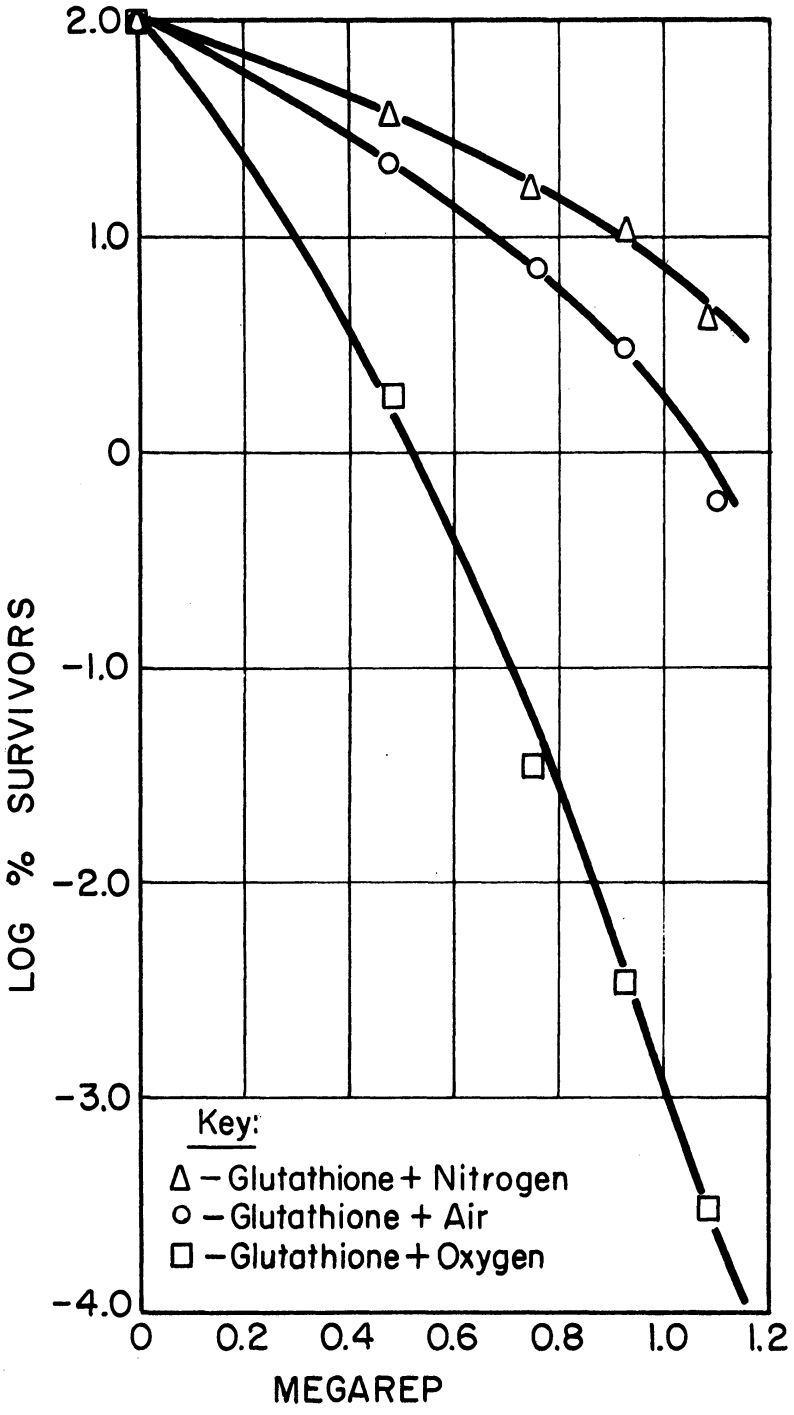

Fig. 6-C. The effect of oxygen tension on the protection afforded C. botulinum 213B spores by glutathione against the lethal effects of gamma rays from cobalt-60.

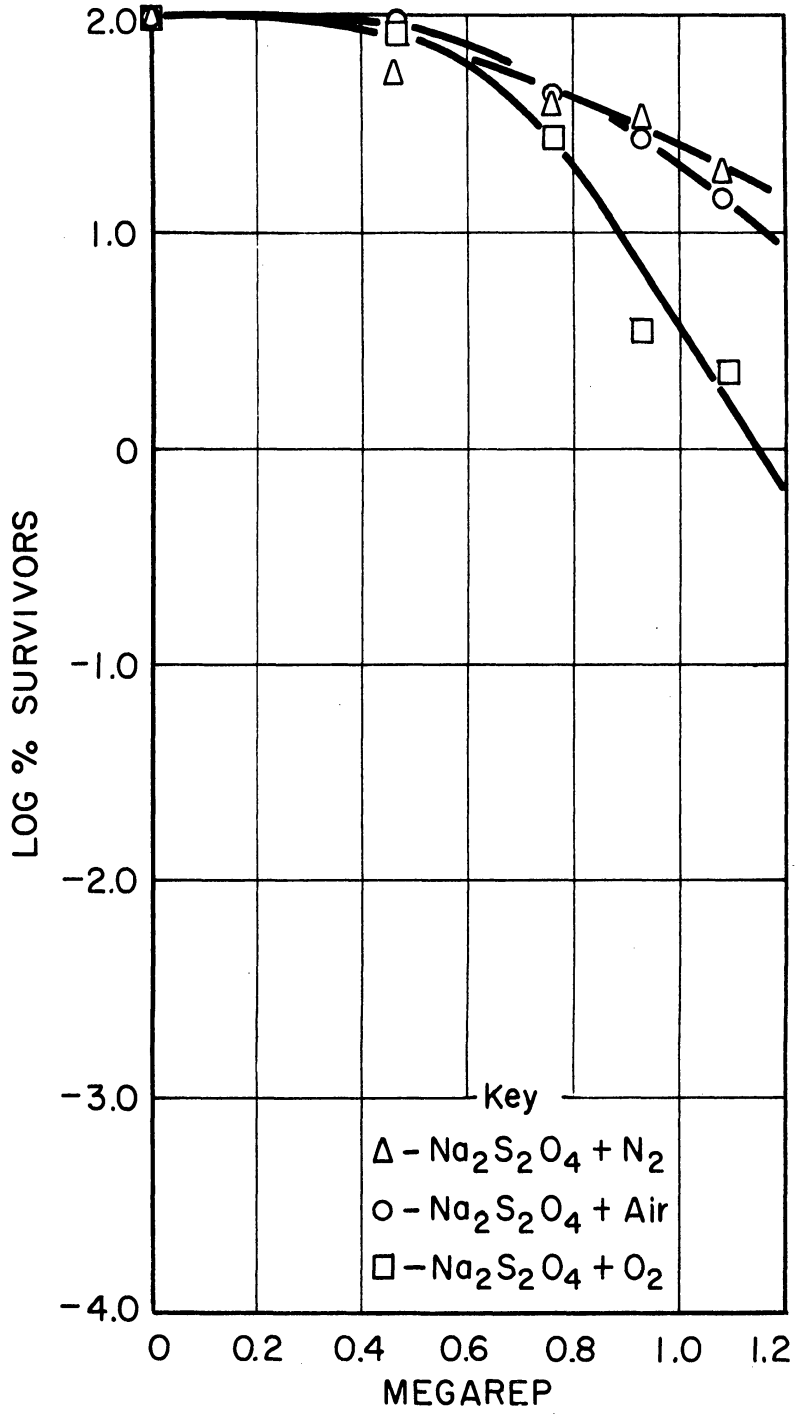

Fig. 7-C. The effect of oxygen tension on the protection afforded C. botulinum 213B spores by sodium hydrosulfite against the lethal effects of gamma rays from cobalt-60.

PHASE III

EFFECTS OF TEMPERATURE DURING IRRADIATION ON THE SURVIVAL OF THE SPORES OF ANAEROBIC BACTERIA

SUMMARY

When anaerobic bacterial spores were irradiated at different temperatures, a temperature zone was found within which the spores were less susceptible to the lethal action of such rays. The temperature zone was different for different spores. However, for the most effective sterilizing results, temperatures of 5°C or below were most desirable for all the spores tested, except where advantage was to be taken of the combined irradiation-heat process.

When the combined irradiation-heating process was used as a sequence of operations, it was found necessary to heat previously irradiated anaerobic bacterial spores above a critical temperature of approximately 95°C before advantage of the combined process was evident.

Also, at temperatures above 95°C, the combined effect of irradiation and heat developed during the irradiation treatment.

INTRODUCTION

The effect of temperature during irradiation on the lethality of gamma radiations for anaerobic bacterial spores becomes important when the irradiation sterilization of food is considered. Since processes involving irradiation at temperatures all the way from -60°C to above 100°C have been proposed, it is necessary to know whether or not these temperature differences are important. Also, does the previously established sensitivity of irradiated spores to subsequent heating apply when radiation and heat are applied simultaneously? If so, this may affect the design of processes for the application of this combined process to food sterilization.

Therefore, our studies in this area of the work have followed three lines. First, the effect of different temperatures during irradiation was observed; second, the effect of irradiation at high temperatures was studied to observe the sensitization effect; and finally, the effect of different holding temperatures following irradiation was noted.

MATERIALS AND METHODS

a. Spores of anaerobic bacteria used in these studies were prepared and used according to techniques described in previously published articles from this laboratory.[1,2]

b. For irradiation the spores were suspended in M/15 phosphate buffer at pH 7.0 and then placed in glass ampoules which accommodated approximately 4 ml of solution. Then the ampoules were heat sealed and placed in an especially designed carrier. The carrier fitted into an apparatus which controlled the temperature ± 1°C during irradiation. Irradiation was carried out in the center well of the large cobalt-60 gamma ray source at the Fission Products Laboratory of The University of Michigan.

RESULTS

Data in Table I-T and Fig. 1-T show that C. botulinum 213B spores are least sensitive to the lethal action of gamma rays in the room temperature range of 15° to 30°C. PA 3679 spores exhibit a similarly narrow temperature range in which they are least sensitive to gamma radiation, but the range is higher, lying between 70° and 100°C.

Table II-T and Fig. 2-T present data to show that spores of C. botulinum 213B are sensitized to the subsequent lethal action of heat at 100°C whether they are preirradiated at 10° or 90°C.

Table III-T and Figs. 3-T and 4-T present data showing the effect of heating previously irradiated spores. Figure 3-T shows that irradiated C. botulinum spores must be heated to a minimum, critical temperature of 80°C before killing occurs. This temperature is also the critical lethal temperature for nonirradiated spores. So, although preirradiation causes C. botulinum spores to be much more rapidly killed by subsequent heating, it does not alter the fact that the spores must be heated to the high temperature of 80°C before the lethal effect of temperature is manifest.

Figure 4-T points out the increased importance of the irradiation sensitization of C. botulinum 213B spores as progressively higher killing temperatures above 80°C are utilized.

Table IV-T and Fig. 5-T show that C. botulinum 213B spores, suspended in M/15 phosphate buffer at pH 7.0, are killed somewhat more rapidly at 4°C than at -70°C; similar results for C. parabotulinum 457 and for PA 3679 spores are shown in Tables V-T and VI-T, respectively. This confirms similar data previously presented for these and other anaerobic bacterial spores.

TABLE I-T

Effect of Temperature During Irradiation with Gamma Rays From Cobalt-60 on the Survival of Anaerobic Bacterial Spores Suspended in M/15 Phosphate Buffer at pH 7.0

Temp., °C	Percent Survivors		
	Dosage Megarep		
	0.550	0.647	0.740
	PA 3679		
5	1.75		1.05
30	4.65	0.697	0.500
56	8.75		0.675
58	12.3		0.229
80	12.7		3.62
85	58.5	20.0	17.2
95	47.5		11.7
	C. botulinum 213B		
-70	8.7	2.8	0.8
- 7	2.0		0.3
5	2.3		0.06
27	11.6		10.5
30	14.0	4.4	0.88
56	10.0	0.3	
58	2.9		0.4
80	2.9		0.14
95	0.16		

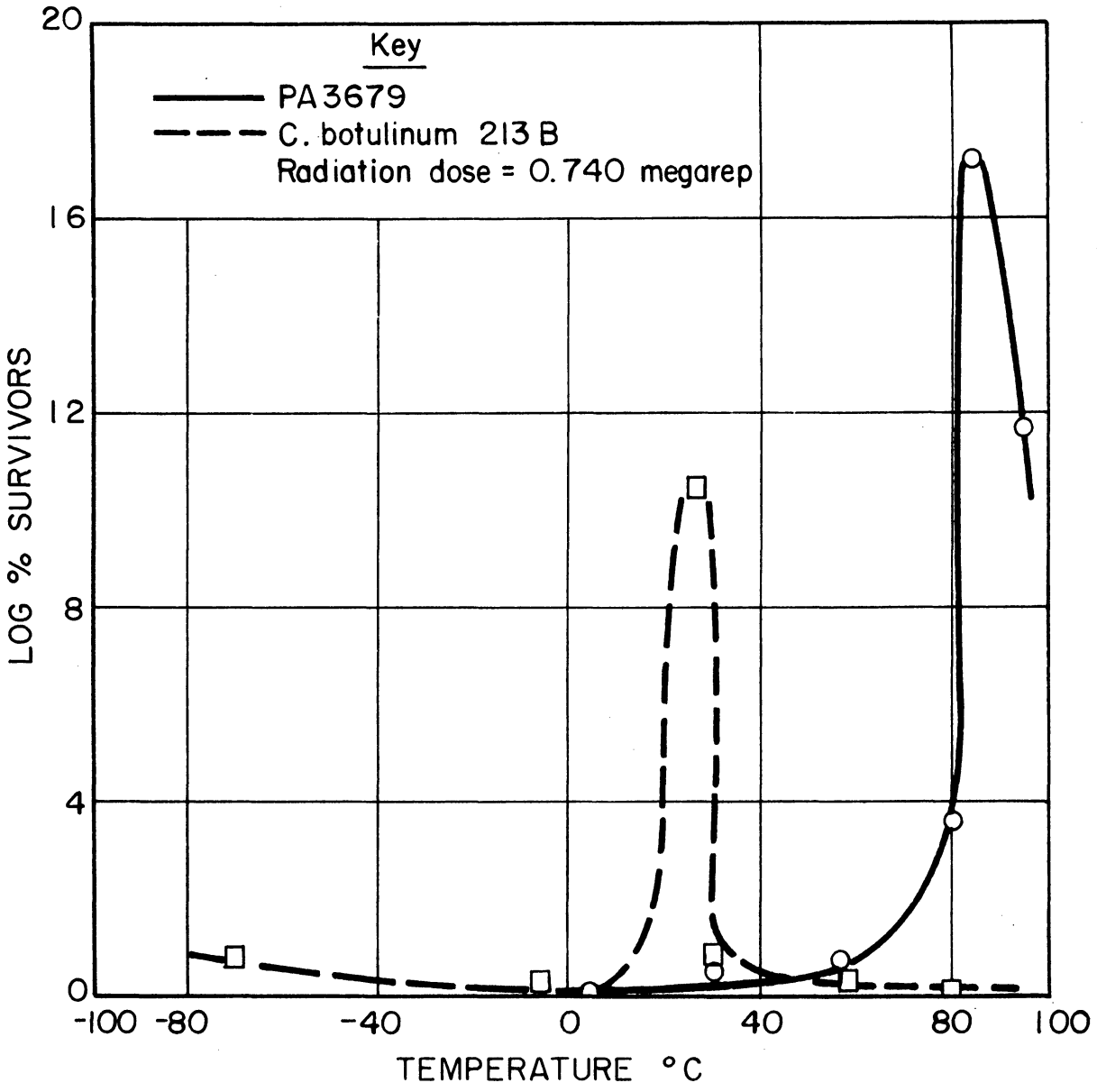

Fig. 1-T. Effect of temperature during irradiation with gamma rays from cobalt-60 on the survival of anaerobic bacterial spores suspended in M/15 phosphate buffer at pH 7.0.

TABLE II-T

Effect of Temperature During Irradiation on the Survival of C. botulinum 213B Spores When They are Subsequently Heated at 100°C, Both Irradiation and Heating Being Carried Out in M/15 Phosphate Buffer at pH 7.0

Minutes at 100°C		Control Spores per ml	% Survivors	Log % Survivors	Irradiated at 10°C			Irradiated at 90°C		
Actual	Effective				Spores per ml	% Survivors	Log % Survivors	Spores per ml	% Survivors	Log % Survivors
0	0	2,200,000	100.0	2.00	230,000	100.0	2.000	600,000	100.0	2.000
10	8.54	1,100,000	50.0	1.70	160,000	69.5	1.842	61,000	10.2	1.009
20	18.54	460,000	20.9	1.32	9,700	4.21	0.624	3,200	0.533	-0.273
30	28.54	85,000	3.86	0.587	1,600	0.695	-0.158	750	0.125	-0.903
40	38.54	22,000	1.00	0.000	360	0.156	-0.807	160	0.0267	-1.574
50	48.54	4,400	0.200	-0.699	30	0.0130	-1.886	13	0.00216	-2.666

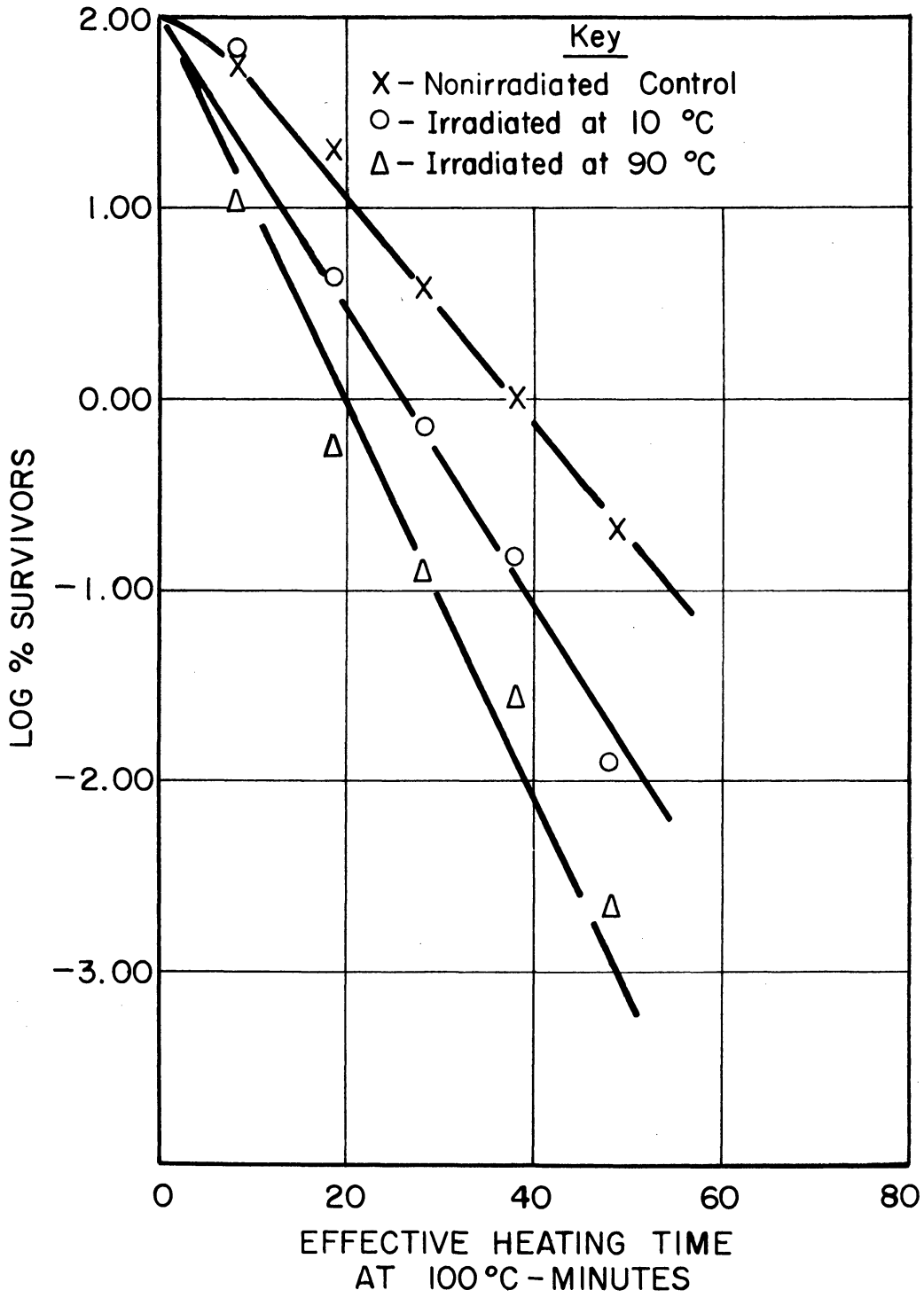

Fig. 2-T. Effect of temperature during irradiation on the survival of C. botulinum 213B spores when they are subsequently heated at 100°C, both irradiation and heating being carried out in M/15 phosphate buffer at pH 7.0.

TABLE III-T

Survival of C. botulinum 213B Spores Suspended in M/15 Phosphate Buffer at pH 7.0, Which Have Been Irradiated at 5°C with Gamma Rays from Cobalt-60 and Then Held for One Hour at the Indicated Temperatures

	Control		100,000 rep		200,000 rep		400,000 rep	
Temp. °C	Spores per ml	Log % Survivors	Spores per ml	Log % Survivors	Spores per ml	Log % Survivors	Spores per ml	Log % Survivors
5	1,050,000	2.000	820,000	2.000	730,000	2.000	370,000	2.000
50	1,050,000	2.000	1,000,000	2.083	630,000	1.940	320,000	1.937
60	930,000	1.947	670,000	1.912	970,000	2.124	240,000	1.813
70	960,000	1.961	620,000	1.879	550,000	1.877	450,000	2.083
80	590,000	1.749	620,000	1.879	720,000	1.984	330,000	1.950
90	630,000	1.778	520,000	1.803	240,000	1.513	106,000	1.457
95	310,000	1.450	11,000	1.335	50,000	0.914	2,800	0.013
100	1,700	-0.791	170	-1.894	40	-2.261	0	

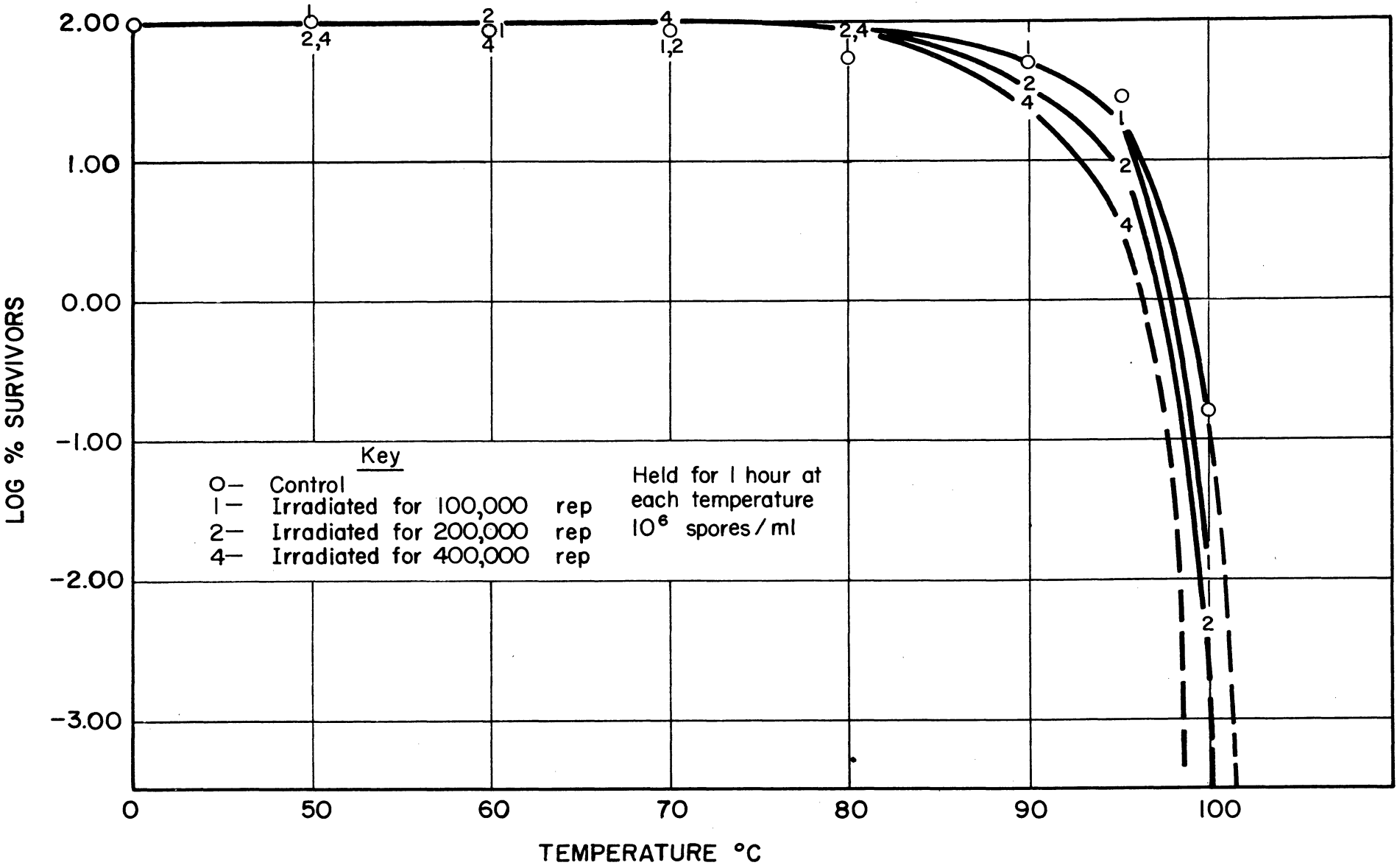

Fig. 3-T. Survival curves for C. botulinum 213B spores suspended in M/15 phosphate buffer at pH 7.0, which have been first irradiated with gamma rays from cobalt-60 and then held for one hour at the indicated temperatures.

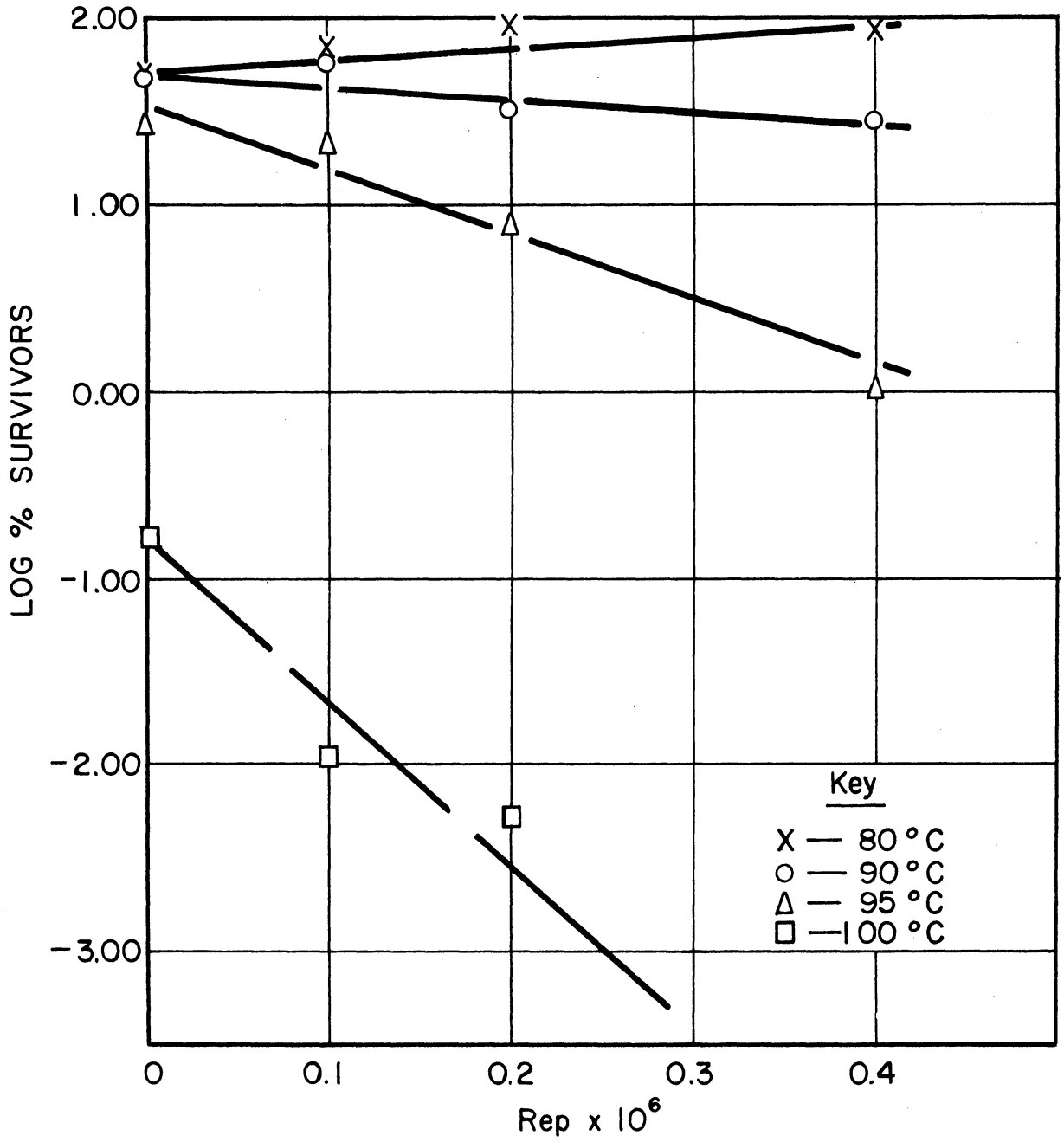

Fig. 4-T. Survival of C. botulinum 213B spores suspended in M/15 phosphate buffer at pH 7.0, which have been irradiated at 5°C with gamma rays from cobalt-60 and then heated for one hour at the indicated temperature.

TABLE IV-T

Effect of Temperature During Irradiation with Gamma Rays from Cobalt-60 on the Survival of C. botulinum 213B Spores Suspended in M/15 Phosphate Buffer at pH 7.0

Dose Rep	Spores per ml	Percent Survivors	Log Percent Survivors
a) Irradiated at 5°C			
0	630,000	100	2.000
340,000	172,000	27.3	1.438
510,000	72,000	11.4	1.057
595,000	11,500	1.83	0.263
680,000	6,100	0.968	-0.014
765,000	760	0.121	-0.917
850,000	370	0.0587	-1.231
b) Irradiated at -70°C			
0	7,500,000	100	2.000
Frozen	9,700,000	129.0	2.076
340,000	5,600,000	57.7	1.761
510,000	1,250,000	12.9	1.110
595,000	710,000	7.33	0.865
680,000	270,000	2.78	0.440
765,000	186,000	1.92	0.283
850,000	35,000	0.361	-0.443

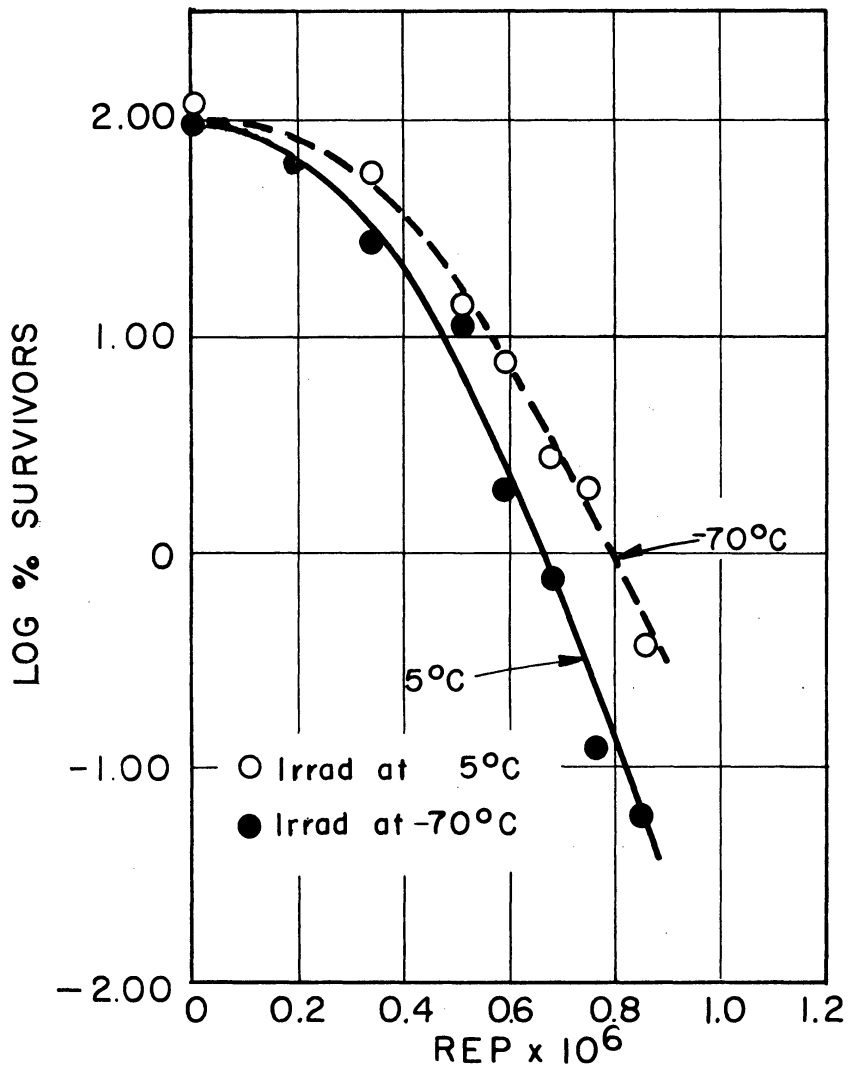

Fig. 5-T. Effect of temperature during irradiation on the survival of C. botulinum 213B spores suspended in M/15 phosphate buffer at pH 7.0

TABLE V-T

Effect of Temperatures During Irradiation with Gamma Rays from Cobalt-60 on the Survival of *C. parabotulinum* 457A Spores Suspended in M/15 Phosphate Buffer at pH 7.0

Dose Rep	Spores per ml	Percent Survivors	Log Percent Survivors
a) Irradiated at 5°C			
0	490,000	100	2.000
340,000	145,000	36.6	1.593
510,000	29,000	5.92	0.772
595,000	9,250	1.89	0.277
680,000	2,550	0.52	-0.284
765,000	950	0.193	-0.714
850,000	145	0.0296	-1.535
b) Irradiated at -70°C			
0	1,900,000	100	2.000
Frozen	2,300,000	121.0	2.083
340,000	1,030,000	44.8	1.651
510,000	385,000	16.7	1.223
595,000	123,000	5.4	0.732
680,000	58,000	2.52	0.401
765,000	17,100	0.774	-0.111
850,000	7,700	0.334	-0.476

TABLE VI-T

Effect of Temperatures During Irradiation with Gamma Rays from Cobalt-60 on the Survival of PA 3679 Spores Suspended in M/15 Phosphate Buffer at pH 7.0

Dose Rep	Spores per ml	Percent Survivors	Log Percent Survivors
a) Irradiated at 5°C			
0	700,000	100	2.000
340,000	230,000	32.8	1.516
510,000	30,000	4.28	0.631
595,000	9,850	1.41	0.149
680,000	2,950	0.237	-0.625
765,000	890	0.127	-0.896
850,000	175	0.025	-1.602
b) Irradiated at -70°C			
0	590,000	100	2.000
Frozen	650,000	110	2.014
340,000	270,000	41.5	1.618
510,000	46,000	7.07	0.849
595,000	46,000	7.07	0.849
680,000	8,600	1.32	0.121
765,000	3,300	0.508	-0.294
850,000	1,200	0.185	-0.733

Data in Table VII-T and Fig. 6-T show that PA 3679 spores in M/15 phosphate buffer at pH 7.0 are not killed in any significant number by heat until a temperature of 105°C is reached. Although such spores that have previously been irradiated with 400,000 or 800,000 rep die in significant numbers when heated to 100°C, it is apparent that a critical temperature of about 95°C is still necessary before irradiated PA 3679 spores become susceptible to killing with heat.

The data in Table VII-T also indicate that although the heat resistance of irradiated PA 3679 spores is reduced considerably above 95°C, until this critical temperature range is reached there is no significant difference between the heat resistances of irradiated and nonirradiated spores.

TABLE VII-T

Effect of Postirradiation Heating for One Hour at Various Temperatures on Previously Irradiated PA 3679 Spores

Temperature, °C	Spores per ml	Percent Survivors	Log Percent Survivors
a) Nonirradiated			
Control	860,000	100	2.000
70	650,000	75.5	1.878
80	660,000	76.7	1.885
90	640,000	74.5	1.872
95	500,000	58.2	1.765
100	580,000	67.5	1.829
105	310,000	34.9	1.543
110	0	0	-
b) Irradiated with 400,000 rep			
Control	150,000	100	2.000
70	170,000	113	2.053
80	160,000	106	2.025
90	162,000	108	2.033
95	175,000	116	2.065
100	134,000	89.5	1.952
105	3,600	2.40	0.380
110	0	0	-
c) Irradiated with 800,000 rep			
Control	1,340	100	2.000
70	200	14.9	1.1732
90	305	22.8	1.358
95	560	41.7	1.620
100	65	4.85	0.686
105	0	0	-
110	0	0	-

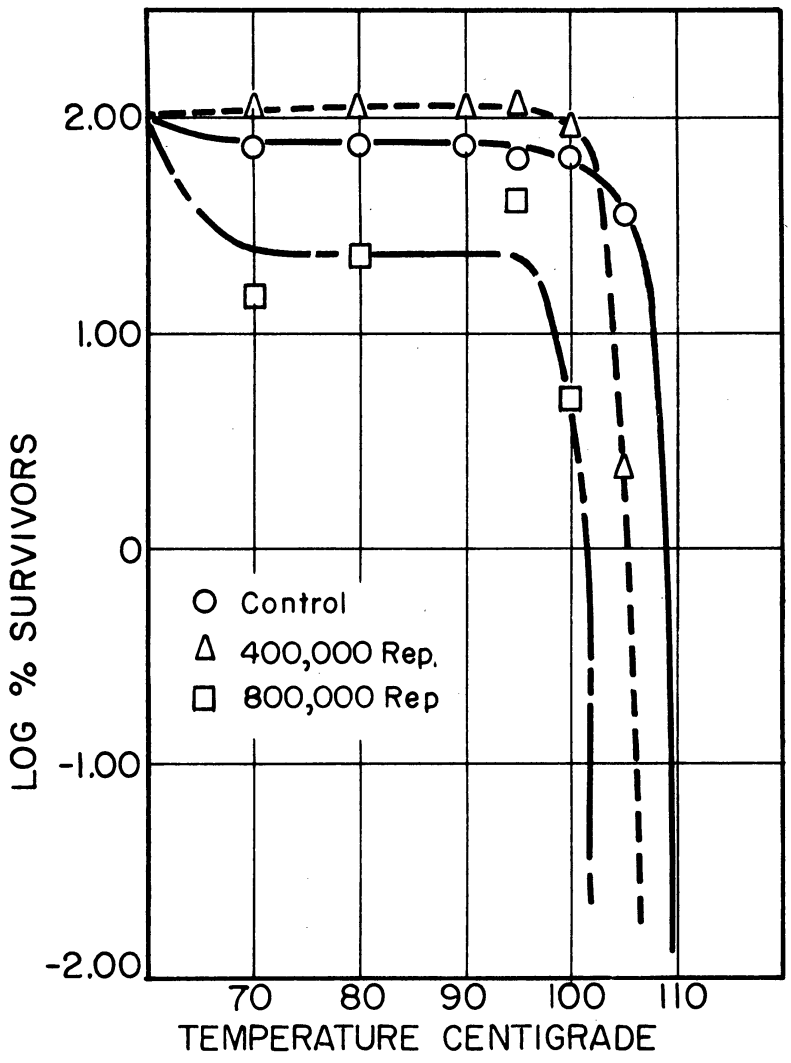

Fig. 6-T. Effect of postirradiation heating for one hour at various temperatures on previously irradiated PA 3679 spores.

DISCUSSION

These data indicate that gamma radiations are most lethal for C. botulinum spores at temperatures below 5°C or above 40°C. In the range of 5° to 40°C, an unexplained protective effect exists. Similarly, PA 3679 spores exhibit a temperature range in which they are less sensitive to gamma radiation; but, for PA 3679 spores this protective temperature range lies between 50° and 95°C. Consequently, it appears to be a fortunate circumstance that the most advantageous irradiation temperature would appear to exist at or below the usual refrigeration temperatures. When advantage is to be taken of the combined process, temperatures above 95°C appear to be desirable.

Data are being collected at present on the effect of temperatures above 80°C on these spores, but there is only the limited amount shown available at this time. Even these limited data indicate that irradiated spores die very rapidly at temperatures of 100°C and above.

It is important to note that any combined heat and irradiation sterilization treatment must be designed only for food that can be heated to at least 95°C since below this critical temperature even irradiated spores are unaffected by heating.

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