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COMBINED USE OF HEAT AND RADIATION TREATMENT FOR STERILIZATION OF FOODS

Period: 7 August 1955 — 7 October 1955

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INTRODUCTION

The work accomplished during this second reporting interval will be presented under four headings, viz.:

(1) Evalution of consecutive irradiation and heat treatment for sterlizing canned meat.

(2) Effects of temperature during irradiation on the survival of bacterial spores.

(3) Effects of chemical composition of the medium during irradiation on the survival of bacterial spores.

(4) Determination of the Z value of irradiated C. botulinum spores.

EVALUATION OF CONSECUTIVE IRRADIATION AND HEAT TREATMENT FOR STERILIZING CANNED MEAT

Previous work has shown that anaerobic bacterial spores are sensitized by preliminary irradiation so that they are killed much more rapidly by subsequent heating than are unirradiated spores. The present study is designed to determine whether the sensitization phenomenon can be successfully applied for reducing the amount of heat required to sterilize canned meat. During the present reporting interval the following has been accomplished.

(1) The autoclave and accessory equipment necessary for following the temperature inside No. 1 picnic tin cans of ground beef has been installed. For this purpose O. F. Eckland thermocouples and a Leeds and Northrup Portable Precision Potentrometer are in use.

(2) A standard operating procedure has been developed for packing, inoculating, irradiating, and heat-processing ground beef in No. 1 picnic cans.

(3) The procedure described in (2) has been used to determine the F_o required to sterilize unirradiated ground beef in No. 1 picnic cans, which is the necessary starting point for this study.

PROCEDURE

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 cans, four of which have been fitted previously with O. F. Ecklund thermocouples. Covers are set loosely on the cans, 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, inoculated with 2 ml of a spore suspension, 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 a 37°C incubator immediately, while the experimental cans are incubated after processing. Processed cans are, of course, quickly cooled to 37°C by immersion in cold water before incubation.

b. Irradiation.—The University of Michigan cobalt-60 source will accommodate 8 No. 1 picnic tin cans at once in the "center well," where the dosage rate is now somewhat less than 200,000 rep per hour. In the warm weather seasons it is necessary to refrigerate the cans during irradiation. This is satisfactorily accomplished with dry ice, but it is more convenient to do this work from late October to April, when the "cave" temperature is near 4°C. Irradiation can also be accomplished outside the source.

c. Heat Processing.—Several false starts were made before a heat-processing technique was developed that yielded reproducible data. 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, thermocouple 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 (1) it represents "hot filling" temperatures of industrial practice, (2) it is not sufficient to cause appreciable killing of spores anywhere in the can during temperature equilibration, and (3) it is high enough to make "come-up" rates to the processing temperature essentially uniform in all the cans.

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

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 at 37°C. The two cans containing thermocouples are again refrigerated; they are used

a second time only.

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

8) F_0 values are calculated according to Halvorson's technique (see Kempe, Applied Microbiology, November, 1955, in press), which is a modification of Bigelow's General Method, apparently similar to that described by Ball in 1928. In addition, the calculations are checked by O. T. Schultz's graphical modification of C. O. Ball's General Method. In these calculations the Z value of irradiated spores is assumed to be 18. However, this is only an assumption, since it is quite possible that irradiation may alter the Z value. Experimental data are being collected to establish the Z value of irradiated spores.

It is concluded from the data shown in Table 1 that ground beef packed in No. 1 picnic tin cans containing approximately 40,000 C. botulinum 213B spores per gram will require an F_0 of 1.0 for sterilization.

Under otherwise similar conditions, except that No. 2 cans were used, it has been reported previously that 3,500,000 rep of gamma radiation from cobalt-60 were required for the sterilization of ground beef canned in a similar manner. (Kempe et al., Applied Microbiology 2, 330-332, 1954.)

These data set the limits for the next phase of this work, which will involve determination of the F_0 required to sterilize canned beef after it has been irradiated with less than 3,500,000 rep of gamma radiation from cobalt-60.

TABLE 1

37°C Incubation Results for No. 1 Picnic Cans of Ground Beef That Have Been Inoculated with C. Botulinum 213B Spores, Irradiated with Gamma Rays from Cobalt-60, and Heat Processed with Increasingly Severe Heat Treatments.

Run C-1

Can size - No. 1 Picnic (2-11/16" x 4")
Product - Ground Beef
Spores - 10,700,000 C. botulinum 213B spores per can
Irradiation - None

F_o		Can No.	Gas Formation	Days to Gas Formation
Inoculated Control		1	+	2
		2	+	2
		3	+	2
		4	+	2
Uninoculated Control		1	0	-
		2	0	-
		3	0	-
		4	0	-
	F_o	1	+	4.5
Can 1	0.26	2	+	5
Can 2	0.31	3	+	5
Avg	0.29	4	+	5
	F_o	5	+	6.5
Can 1	1.00	6	+	7
Can 2	1.00	7	+	6
Avg	1.00	8	+	11
	F_o	9	+	6.5
Can 1	0.80	10	+	7.5
Can 2	1.25	11	+	7.5
Avg	1.02	12	+	6.5
	F_o	13	+	6.5
Can 1	0.53	14	+	5.5
Can 2	0.57	15	+	7.5
Avg	0.56	16	+	5.5

Result: F_o required is more than, but very close to, 1.0.

Remarks: Used preheat water-bath temperature at 210°F and sterilizing bath at 230°F.

TABLE 1 (Cont.)

Run C-2

Can size - No. 1 Picnic (2-11/16" x 4")
Product - Ground Beef
Spores - 7,200,000 C. botulinum 213B per can
Irradiation - None

F_o		Can No.	Gas Formation	Days to Gas Formation
Inoculated Control		1	+	1.5
		2	+	1.5
		3	+	1.5
		4	+	1.5
Uninoculated Control		1	0	-
		2	0	-
		3	0	-
		4	0	-
	F_o	1	+	3.5
Can 1	0.28	2	+	3.5
Can 2	0.39	3	+	3.5
Avg	0.34	4	+	3.5
	F_o	5	0	-
Can 1	0.70	6	0	-
Can 2	0.73	7	0	-
Avg	0.72	8	+	3.5
	F_o	9	+	3.5
Can 1	0.75	10	+	4.5
Can 2	1.01	11	+	4.5
Avg	0.88	12	0	-
	F_o	13	0	-
Can 1	1.24	14	0	-
Can 2	1.29	15	0	-
Avg	1.27	16	0	-

Result: F_o required is between 0.8 and 1.3.

Remarks: Used preheat water-bath temperature at 210°F and sterilizing bath at 230°F.

TABLE 1 (Concl.)

Run C-3

Can size - No. 1 Picnic (2-11/16" x 4")
Product - Ground Beef
Spores - 10,700,000 C. botulinum 213B spores per can
Irradiation - None

F_o		Can No.	Gas Formation	Days to Gas Formation
Inoculated Control		1	+	1.5
		2	+	1.5
		3	+	1.5
		4	+	1.5
Uninoculated Control		1	0	-
		2	0	-
		3	0	-
		4	0	-
	F_o	1	+	6
Can 1	0.36	2	+	6
Can 2	0.49	3	+	6
Avg	0.43	4	+	6
	F_o	5	0	-
Can 1	0.93	6	0	-
Can 2	0.93	7	0	-
Avg	0.93	8	0	-
	F_o	9	0	-
Can 1	1.18	10	0	-
Can 2	1.53	11	0	-
Avg	1.36	12	0	-
	F_o	13	0	-
Can 1	1.59	14	0	-
Can 2	1.82	15	0	-
Avg	1.71	16	0	-

Result: F_o required is between 0.4 and 0.9.

Remarks: Used preheat water-bath temperature at 190°F and sterilizing bath at 230°F.

EFFECTS OF TEMPERATURE DURING IRRADIATION ON THE SURVIVAL OF BACTERIAL SPORES

During the past few months, three lines of investigation have been followed to evaluate some of the combined effects of temperature and irradiation on anaerobic bacterial spores suspended in M/15 phosphate buffer at pH 7.0. First, we have investigated the effect of temperature during irradiation on the survival of these spores; second, we have been interested to learn whether irradiation at a high temperature affected the sensitization of spores to the subsequent lethal action of heat; and third, we have tested the effect of different holding temperatures following irradiation of the spores.

Data in Table 2 and Fig. 1 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 3 and Fig. 2 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. The slight difference in sensitivity indicated between spores irradiated at these temperatures may be more apparent than real.

Table 4 and Figs. 3 and 4 present data showing the effect of heating previously irradiated spores. Figure 3 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 unirradiated 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 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 2

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		

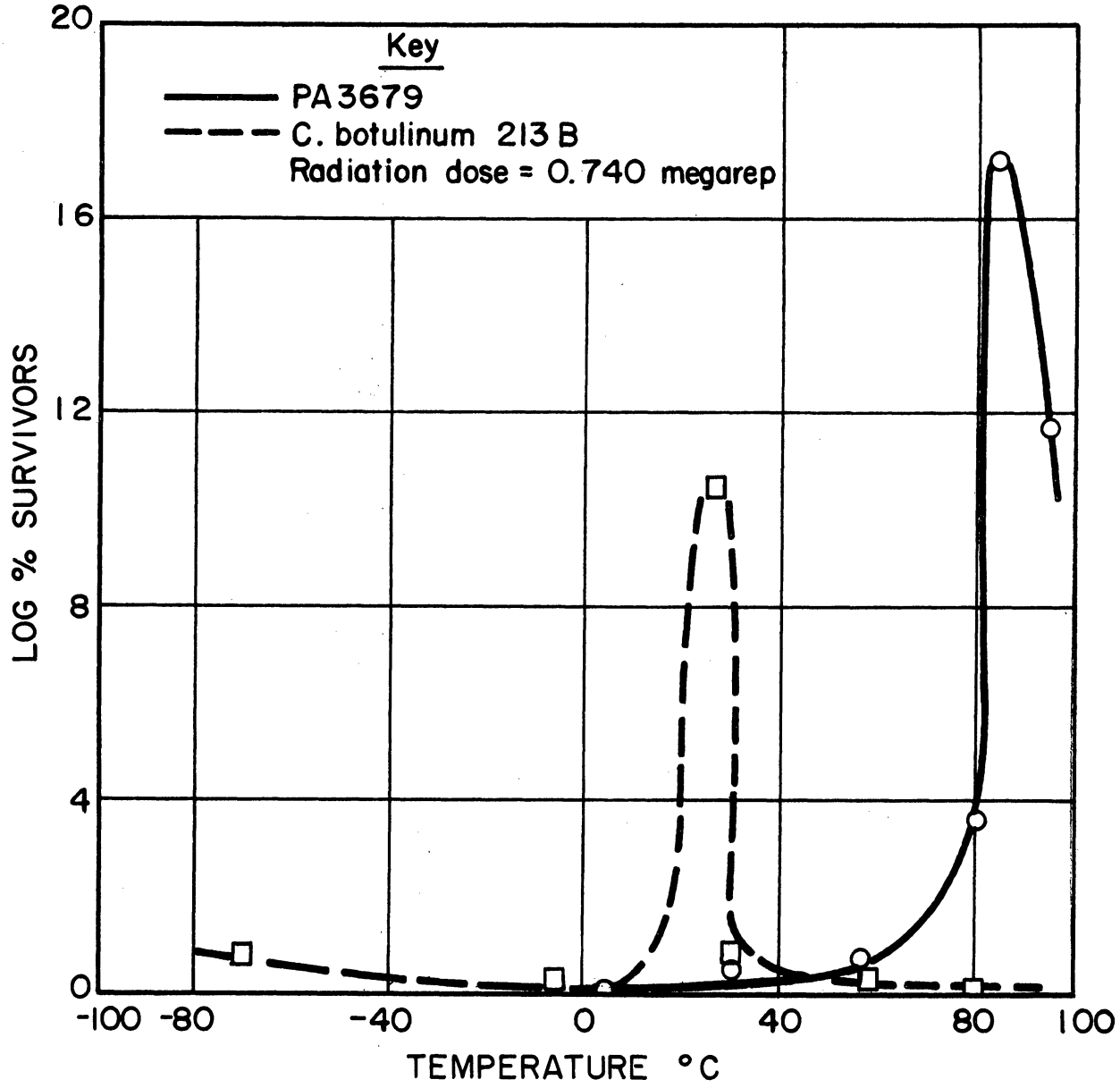

Fig. 1. Effect of temperature during irradiation with gamma rays from cobalt-60 on the survival of anaerobic bacterial spores suspended in M/15 phosphate buffer at pH 7.0.

TABLE 3

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

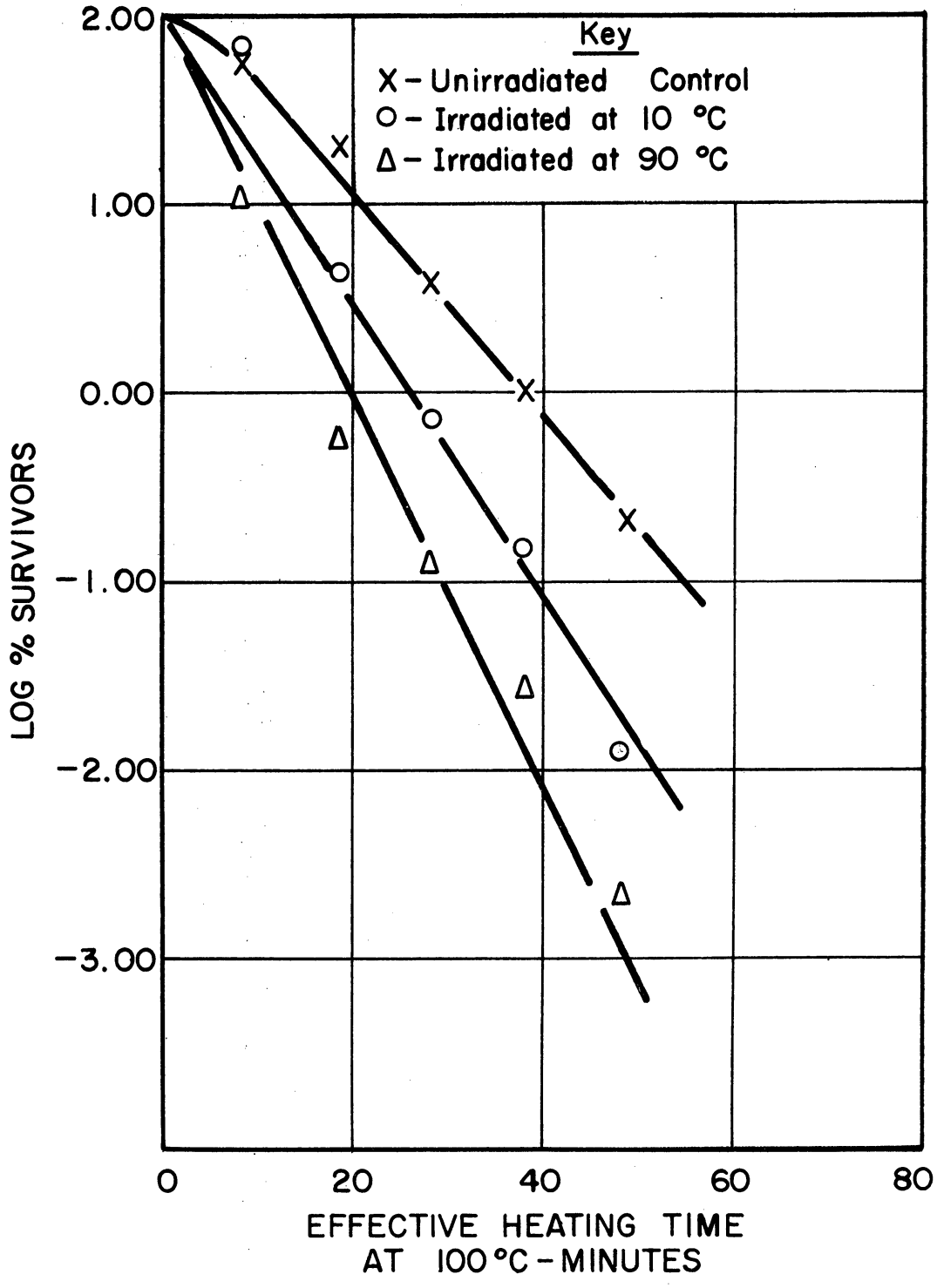

Fig. 2. Effect of temperature during irradiation on the survival of C. botulinum 213B spores when they are subsequently heated at 100°C, both irradiation and heating being carried out in M/15 phosphate buffer at pH 7.0.

TABLE 4

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 Temperature

	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	

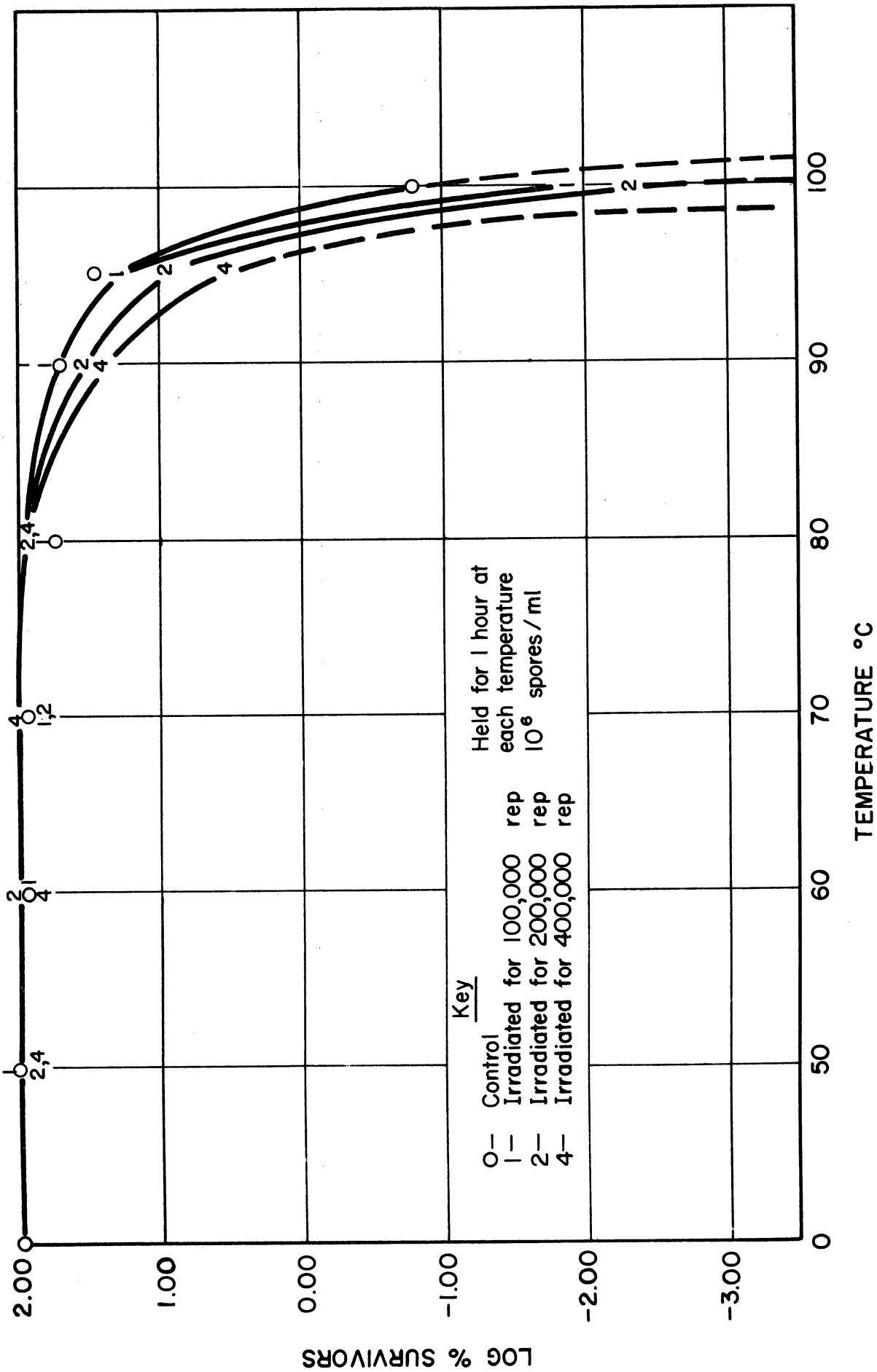

Fig. 3. Survival curves for C. botulinum 213B spores suspended in M/15 phosphate buffer at pH 7.0, which have been first irradiated with gamma rays from cobalt-60 and then held for one hour at the indicated temperatures.

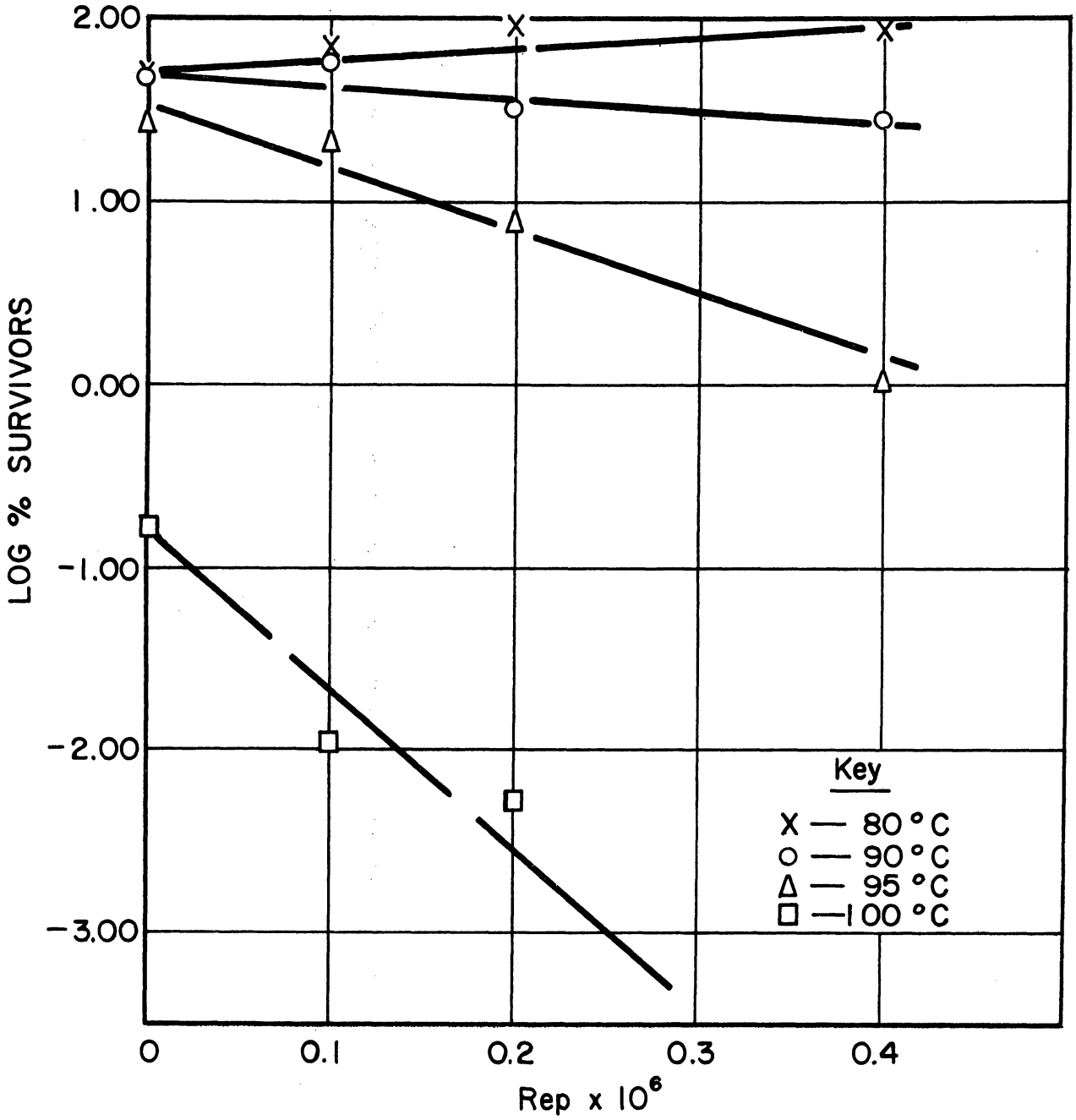

Fig 4. Survival of C. botulinum 213B spores suspended in M/15 phosphate buffer at pH 7.0, which have been irradiated at 5°C with gamma rays from cobalt-60 and then heated for one hour at the indicated temperature.

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

SUMMARY

It has been previously reported that certain chemicals present in the suspending medium during irradiation of C. botulinum 62A spores, reduce the lethality of gamma radiation from cobalt-60 for these spores. This work has been extended, using C. botulinum 213B and some additional chemical agents.

It has been found that the chemicals that reduce the lethality of gamma radiation for spores of C. botulinum 62A also reduce the lethality of such radiation for spores of C. botulinum 213B.

Sodium hydrosulfite and glutathione have been tested with both strains of C. botulinum spores and have been observed to be quite effective in reducing the lethality of gamma radiation, sodium hydrosulfite being the most active protective agent tested so far.

This established fact, that many chemicals reduce the lethality of gamma radiation for anaerobic bacterial spores, emphasizes the conclusion that caution must be exercised in stating the lethal dose of irradiation to be used for a specific food or in any particular application.

METHODS AND MATERIALS

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 an identical manner to that previously described for C. botulinum 213B spores.

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.

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.

Control

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

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

The data taken during this reporting interval with C. botulinum 213B spores substantiate previous data 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 5 and 6 and in the companion figures 5 and 6.

TABLE 5

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

Irradiation 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 6

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

Irradiation 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

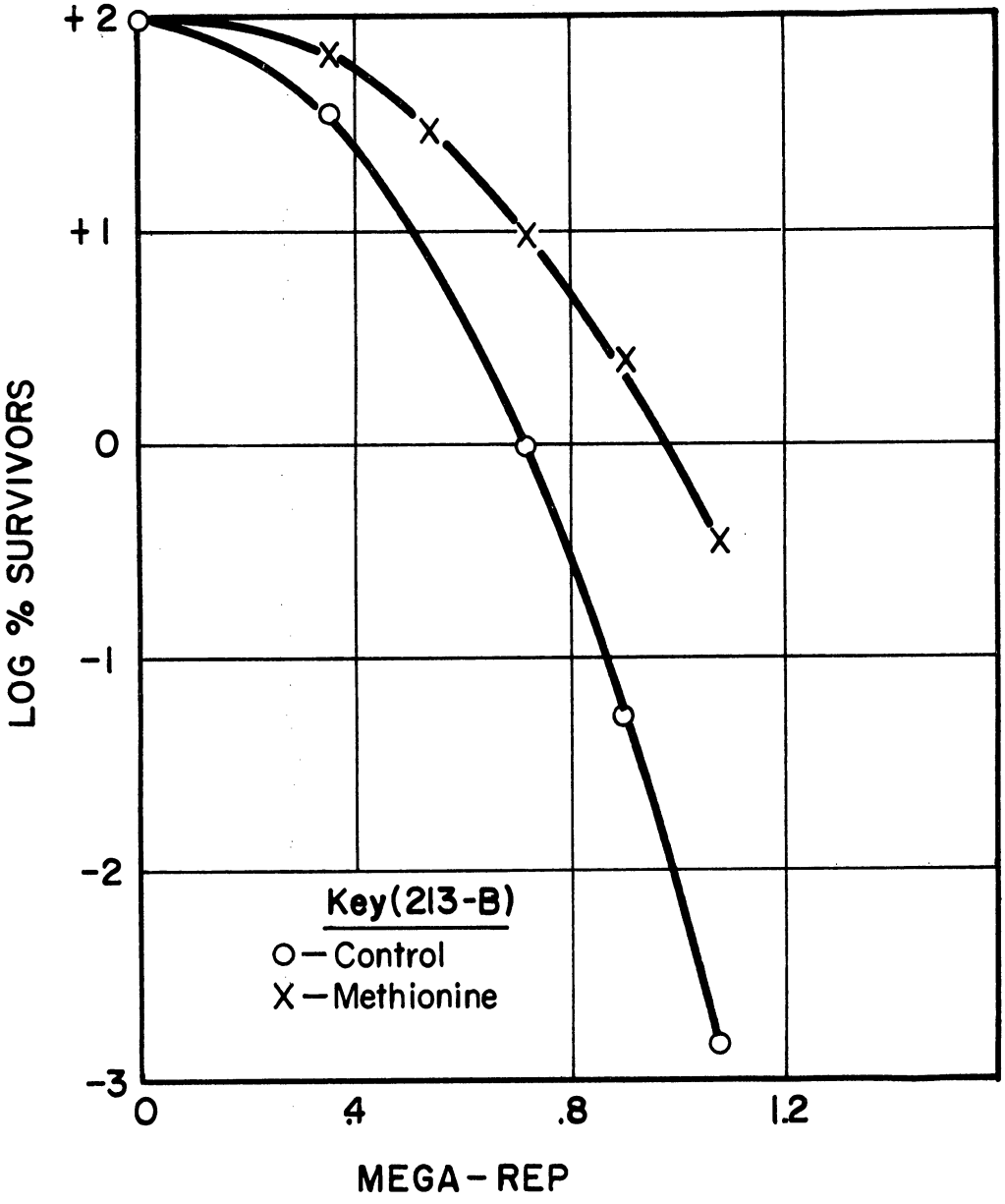

Fig. 5. Effect of methionine in M/15 phosphate buffer of pH 7.0 on the lethality of gamma radiation from cobalt-60 for the spores of C. botulinum 213B.

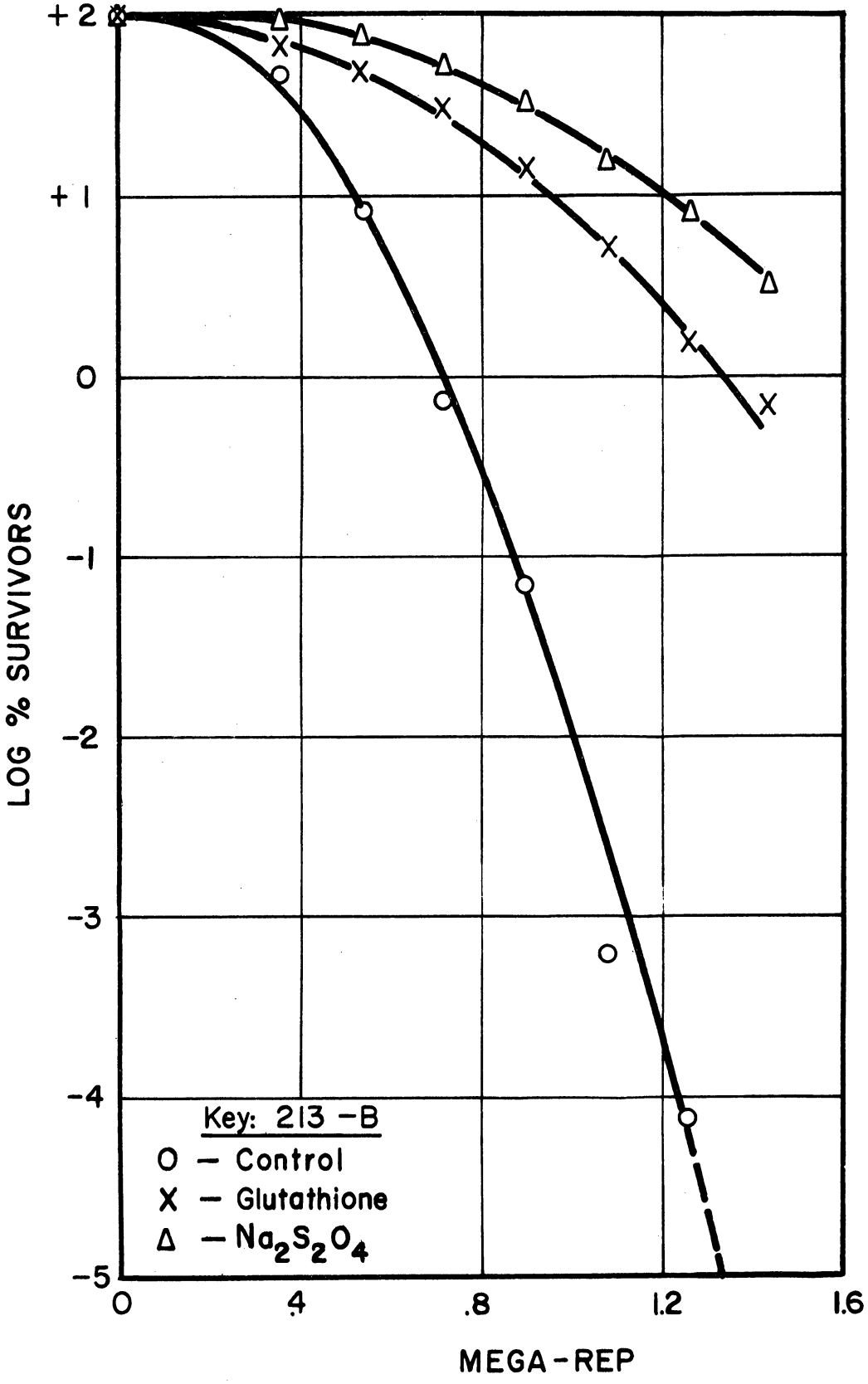

Fig. 6. Effects of glutathione and sodium hydrosulfite added to M/15 phosphate buffer at pH 7.0 on the lethality of gamma radiation from cobalt-60 for the spores of C. botulinum 213B.

PRELIMINARY COMPARISON OF THE Z VALUE OF IRRADIATED AND UNIRRADIATED SPORES OF PA 3679

An investigation designed to determine whether a difference exists in the Z value of irradiated and unirradiated spores was deemed necessary since (1) all calculations made thus far in studies pertaining to inactivation of spores which had been exposed to irradiation were made on the assumption that the Z value was 18, (2) any appreciable change in the Z values caused by irradiation would markedly affect the rationale of the methods now employed in calculating the F_0 values when combined heat and irradiation techniques are used. Consequently, preliminary investigations are being conducted to determine the Z value of irradiated and unirradiated spores.

MATERIALS AND METHODS

Spores of PA 3679 were obtained by growth in pork-extract broth for 3 weeks at 30°C. Cells were harvested at 4°C and washed 10 times in sterile distilled water. They were resuspended, and vegetative cells were inactivated by heat shocking at 99°C for 5 minutes. The concentrated spore suspension was diluted in neutral phosphate buffer until it contained approximately 10^6 spores/ml. Four ml of such a spore suspension were introduced aseptically into 5-ml neutral glass vials which were then sealed in an oxygen flame. One group of vials was exposed to gamma irradiation so that the spores received a dosage of 250,000 rep while another group was not irradiated. Both series of vials were subsequently heat treated at temperatures between 100°C and 120°C for varying lengths of time. This was accomplished by immersing the vials in a constant-temperature oil bath and removing samples at the desired time intervals. A correction was made for the time required for the contents of the vials to reach the temperature of the bath.

The entire contents of a vial was inoculated into a Prickett tube containing pork-extract agar, layered with agar and paraffin, and allowed to incubate for 3 weeks. Prolonged incubation was found necessary since the severe heat shocking delayed the germination of the viable spores. Survival points were then obtained as indicated by growth or no growth in the Prickett tubes.

RESULTS

Tables 7 and 8 and Figs. 7 and 8 show the composite data of two independent runs. Z values were obtained by plotting the logarithm of the time required to inactivate all the spores against the temperature in degrees centigrade. Thermal inactivation curves were drawn in such a way that no points indicating growth lie above the line.

The Z values obtained are essentially the same as those reported by Reed *et al.** The value of 23 found for irradiated spores was approximately 2 units higher than the value of 21.3 found for nonirradiated spores. Investigations will be continued to determine whether or not this difference is statistically significant.

CONCLUSION

Preliminary investigations indicate a slight difference for Z values of irradiated and unirradiated spores. Experiments will be continued until valid statistical analysis can be made. Future investigations will also include determinations of the Z value of spores exposed to various levels of gamma irradiation.

*Reed, J. M., C. W. Bohrer, and E. S. Cameron, Food Research, 16, 383-408 (1951).

TABLE 7

Z Value Determination for Irradiated PA 3679 Spores Suspended in M/15 Phosphate Buffer at pH 7.0 Using 1.8 x 10^6 Spores Per Tube

Heating Time (min)	Growth on Incubation at 30°C	Heating Time (min)	Growth on Incubation at 30°C	Heating Time (min)	Growth on Incubation at 30°C
	125°C		120°C		115°C
3	+	4	+	12	+
4	+	6	+	14	+
5	+	8	+	16	+
6	-	10	+	18	+
7	-	12.5	-	20	+
				22	+
				24	+
				26	+
				28	-
				30	-
	110°C		105°C		
20	+	90	+		
25	+	100	+		
30	+	110	-		
35	+	130	+		
40	+	140	+		
45	+	160	+		
50	+	170	+		
55	+	180	+		
60	+	190	-		
65	+	200	-		
70	+	210	-		
75	-				
80	-				
85	-				

TABLE 8

Z Value Determination for Nonirradiated Spores of PA 3679 Suspended in M/15 Phosphate Buffer at pH 7.0 Using 4 x 10^6 Spores Per Tube

Heating Time (min)	Growth on Incubation at 30°C*	Heating Time (min)	Growth on Incubation at 30°C*	Heating Time (min)	Growth on Incubation at 30°C*
	125°C		120°C		115°C
5	+	10	+	10	+
6	-	11	+	15	+
7	-	12	+	19	+
8	-	13	-	20	+
9	-	14	-	25	+
10	-	15	-	30	+
11	-	16	-	35	+
12	-	17	-	40	-
				45	-
	110°C		105°C		
40	+	100	-?		
45	+	150	+		
50	+	175	+		
55	+	225	+		
60	+	230	-		
65+	+	240	+		
70	+	250	-		
80	-	260	-		
90	-	270	+		
100	-	280	-		
105	-	300	-		

* + growth
- no growth

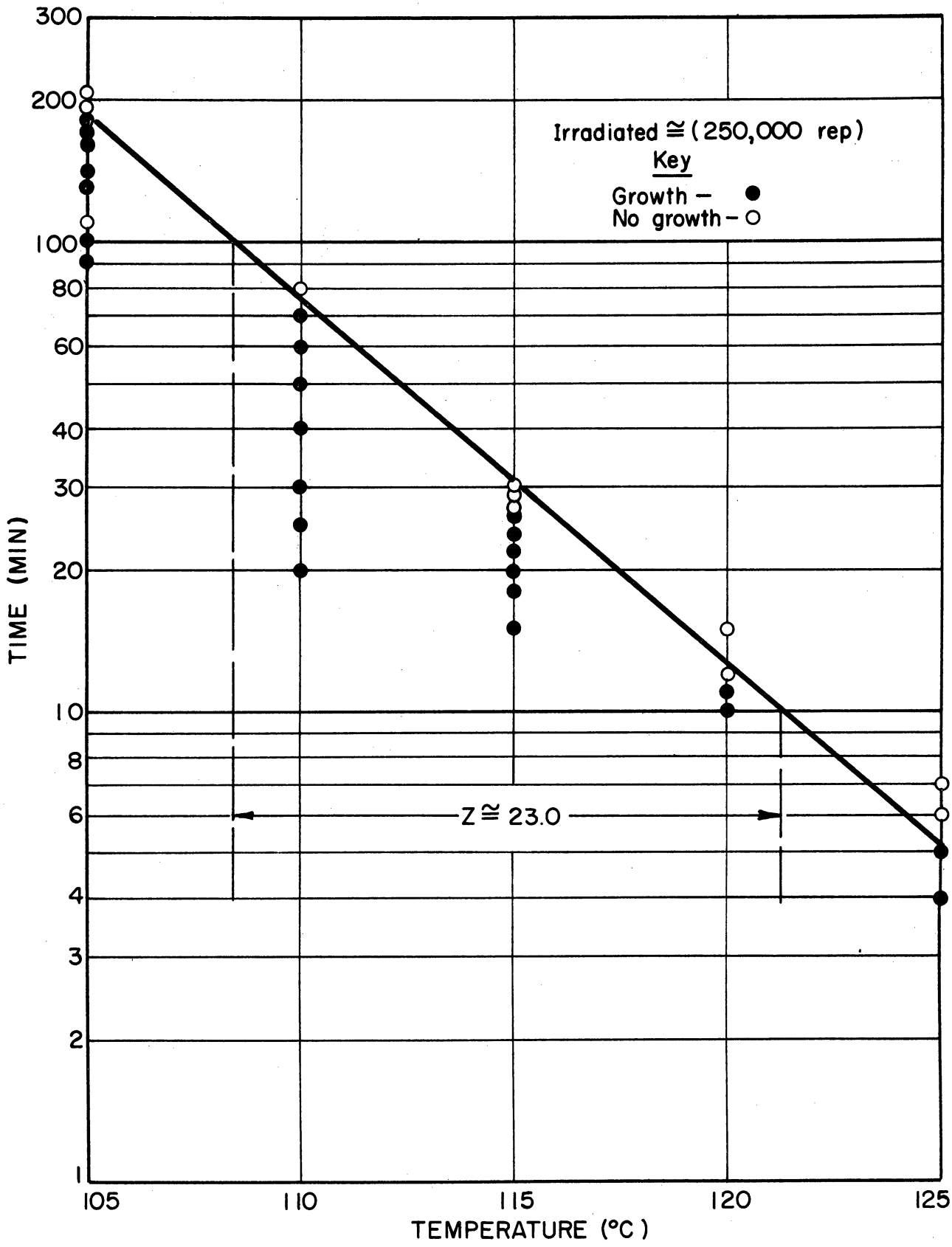

Fig. 7. Thermal death time curve for irradiated PA 3679 spores suspended in M/15 phosphate buffer at pH 7.0.

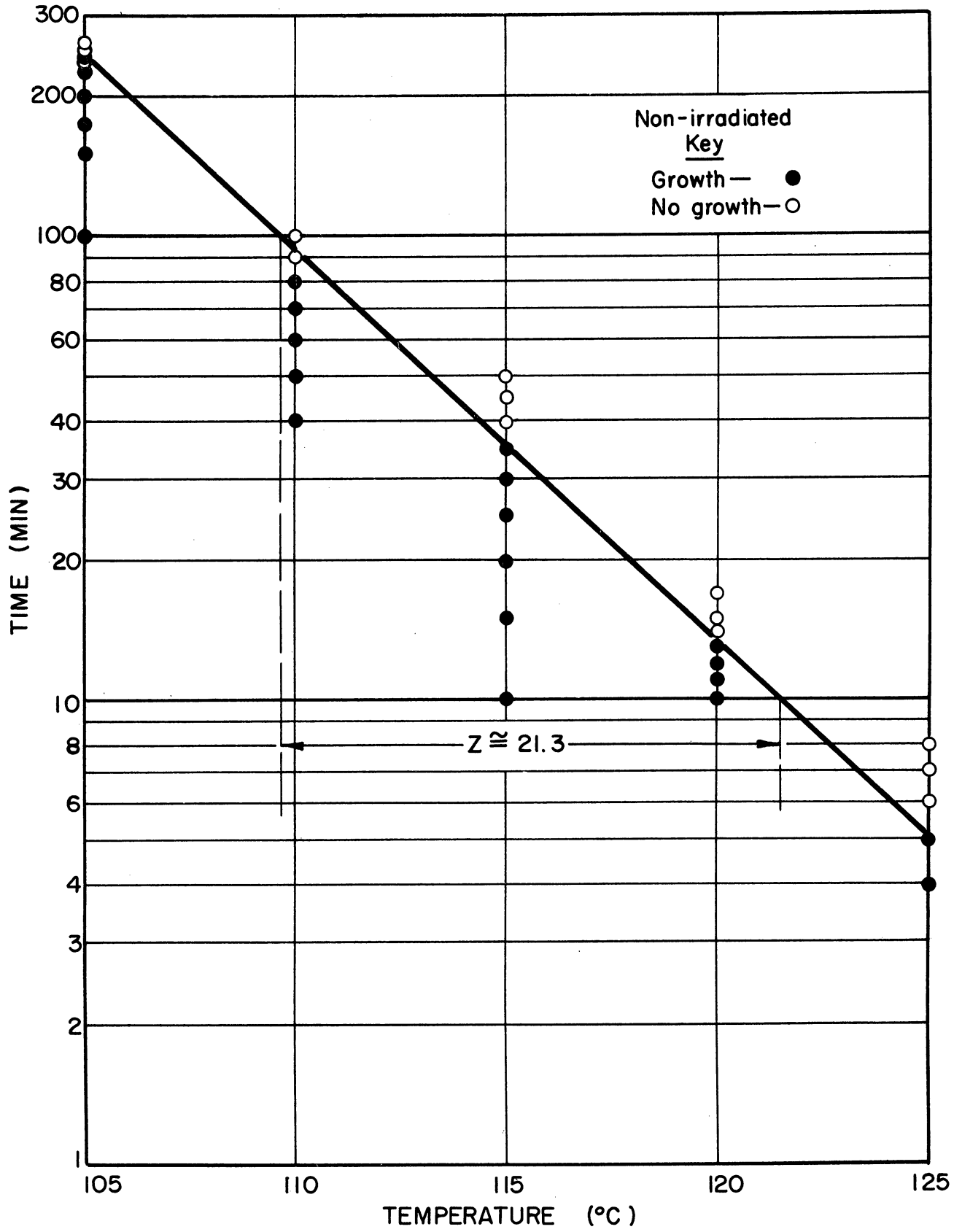

Fig. 8. Thermal death time curve for nonirradiated PA 3679 spores suspended in M/15 phosphate buffer at pH 7.0.