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THE UNIVERSITY OF MICHIGAN
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Progress Report No. 4

COMBINED USE OF HEAT AND RADIATION
TREATMENT FOR STERILIZATION OF FOODS

Period: 7 December 1955 — 7 February 1956

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Project 2391

QUARTERMASTER RESEARCH AND DEVELOPMENT COMMAND
NATICK, MASSACHUSETTS
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CONTRACT RESEARCH PROJECT REPORT

QUARTERMASTER FOOD AND CONTAINER INSTITUTE
FOR THE ARMED FORCES, CHICAGO

Hq, QM Research and Development Command,

QM Research and Development Center, Natick, Mass.

The University of Michigan
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Treatment for Sterilization of Foods

SUMMARY

The first series of tests to determine the value of the combined use of heat and irradiation treatment for the sterilization of canned meat has been completed. Using an inoculum of approximately 5,000,000 C. botulinum 213B spores per No. 1 picnic can of meat, it was found:

1) Approximately 1,000,000-rep preirradiation is required before a significant effect of irradiation is noted on the F_0 value required for subsequent heat sterilization of the canned meat.

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2) Following irradiation with from 1,200,000 to 1,500,000 rep of gamma radiation, the F_0 for sterilizing canned meat under the above conditions is lowered from a value of 1.0 to less than 0.25.

Work on the effects of chemicals and temperatures during irradiation on the lethality of gamma radiation for anaerobic bacterial spores has continued but has been largely confined to confirming data already reported. Thus it has been shown that glutathione and sodium hydrosulfite protect PA 3679 spores in the same manner as they do C. botulinum spores. And the pH of the medium, in the range of 3.2 to 8.4, does not influence the lethality of gamma radiation for C. botulinum spores.

EVALUATION OF CONSECUTIVE IRRADIATION AND HEAT TREATMENT FOR STERILIZING CANNED MEAT

The methods and equipment for this study were presented in Progress Report No. 3. Briefly they involve preparation of inoculated packs of lean ground beef in No. 1 picnic tin cans which are closed while hot in order to develop vacua. The cans are then irradiated in the large cobalt-60 source at The University of Michigan while held at a temperature of about 4°C. They are next processed in a steam autoclave and finally incubated at 37°C. Sufficient F_0 values are used to permit bracketing that F_0 value which is required just to sterilize all four cans. Sterility is routinely determined by observation for swelling of the cans during incubation.

In Fig. 1 and Table I it is shown that an F_0 of 1.0 developed at the coldest spot in the can during processing was required to sterilize ground beef that had been inoculated with approximately 5,000,000 C. botulinum 213B spores per can. This value was reduced to less than 0.25 following irradiation with 1,200,000 rep. However, little reduction of the F_0 was noticed until 1,000,000 rep were used.

At present, runs are being made with 250 rather than 5,000,000 C. botulinum 213B spores per can.

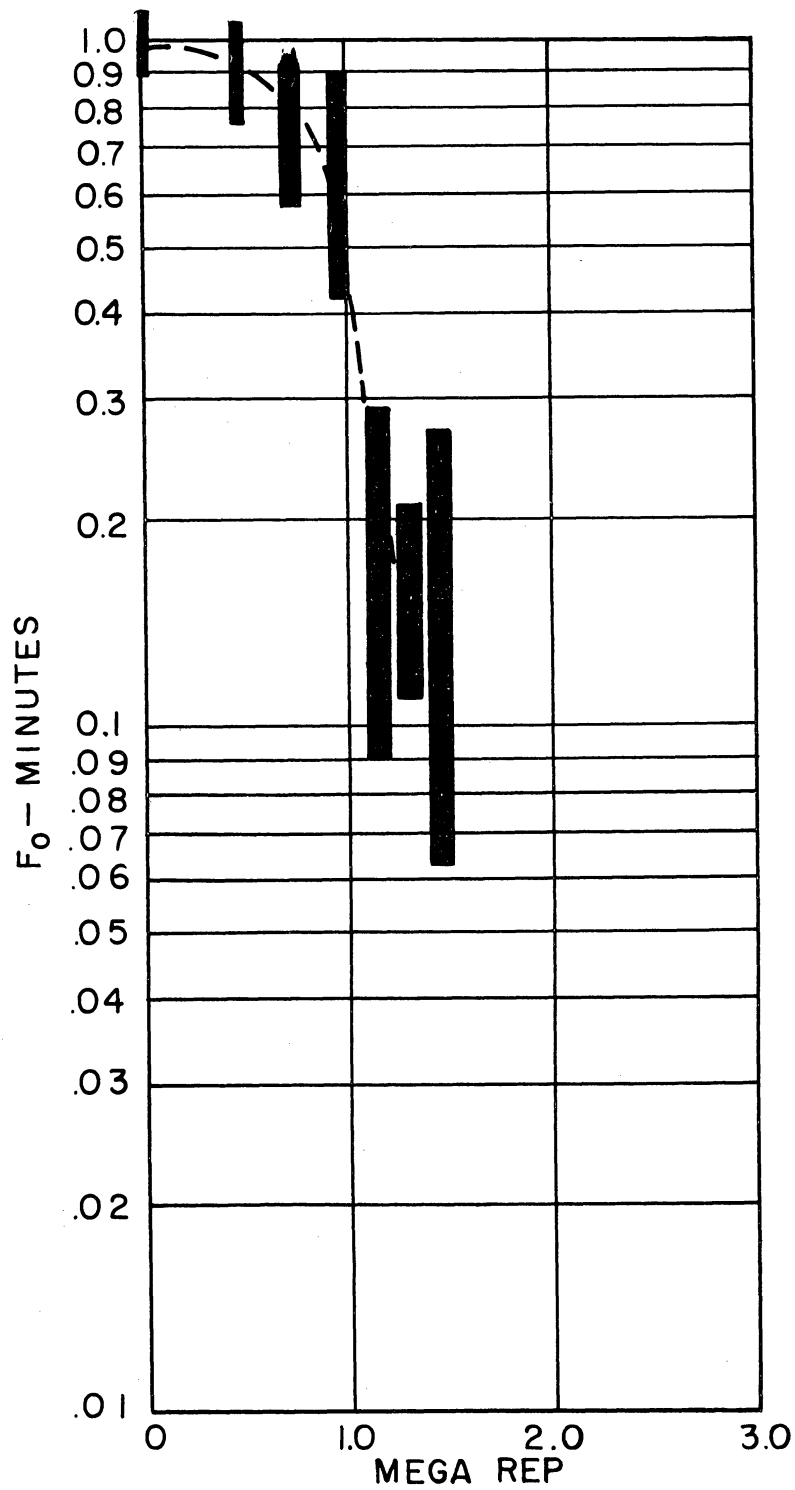


Fig. 1. F_0 required to sterilize ground beef packed in No. 1 picnic tin cans, inoculated with approximately 5,000,000 C. botulinum 213B spores per can and irradiated with gamma rays from cobalt-60 before heat processing.

TABLE I - F_0 Required to Sterilize Ground Beef Packed in No. 1 Picnic Tin Cans, Inoculated with Approximately 5×10^8 *C. botulinum* 213B Spores and Then Irradiated with Gamma Rays from Cobalt-60 Before Heat Processing.

Run CB-8: Can Size - No. 1 Picnic (211 x 400)
 Product - Ground Beef
 Inoculum - 5,000,000 *C. botulinum* 213B
 Preirradiation - 500,000 rep

F_0	Can No.	Gas Formation	Days to Gas Formation
Uninoculated Control	1	+	2
	2	-	
	3	-	
	4	-	
Inoculated Control	1	+	2
	2	+	2
	3	+	2
	4	+	2
F_0 Can 1, 1.40 Can 2, 0.85	1	-	
	2	-	
	3	-	
	4	-	
Can 1, 0.745 Can 2, 0.639	5	-	
	6	-	
	7	-	
	8	-	
Can 1, 1.05 Can 2, 0.73	9	-	
	10	-	
	11	-	
	12	-	
Can 1, 0.881 Can 2, 0.540	13	-	
	14	-	
	15	-	
	16	-	
Nonirradiated Controls	17	-	
	18	-	
Can 1, 0.86	19	-	
	20	-	
Can 1, 1.18	21	-	
	22	-	
	23	-	
	24	-	

Conclusion: None

TABLE I (Continued)

Run 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

F ₀	Can No.	Gas Formation	Days to Gas Formation
Uninoculated 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
	6	+	3
Can 2, 0.11	7	+	3
	8	-	-
Can 1, 0.013	9	+	2
	10	+	2
Can 2, 0.019	11	+	3
	12	+	3
Can 1, 0.062	13	+	5
	14	+	4
Can 2, 0.081	15	-	
	16	-	

Conclusion: After 1,500,000 rep, sterility dosage is more than an F₀ of 0.11.

TABLE I (Continued)

Run CB-10: Can Size - No. 1 Picnic
 Product - Ground Beef
 Inoculum - 6,000,000 C. botulinum 213B spores per can
 Preirradiation - 500,000 rep

F_0	Can No.	Gas Formation	Days to Gas Formation	
Uninoculated Controls	1	+	11	
	2	-		
	3	-		
	4	-		
Inoculated Controls	17	+	2.5	
	18	+	2.5	
	19	+	2.5	
	20	+	2.5	
Can 1, 0.43 Can 2, 0.26	1	+	5	
	2	+	6	
	3	+	5	
	4	+	6	
Can 1, 0.56 Can 2, 0.51	5	+	6	
	6	+	6	
	7	-	-	
	8	+	6	
Can, 1.06	9	-		
	10	-		
	11	-		
	12	-		
Can 1, 0.84 Can 2, 0.77	13	+	9	
	14	-		
	15	-		
	16	+	7	
Nonirradiated Controls	20	+	7	
	21	+	7	
	0.81 0.68	22	+	6
		23	+	7
0.98	24	-		
	25	-		
	26	-		
	27	+	8	

Conclusion: With 500,000 rep preirradiation the sterility dosage lies between F_0 values of 0.77 and 1.06.

TABLE I (Continued)

Run 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

F ₀	Can No.	Gas Formation	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 Can 2, 0.206	1	+	5
	2	-	
	3	-	
	4	-	
Can 1, 0.125 Can 2, 0.072	5	+	3
	6	-	-
	7	-	-
	8	+	3
Can 1, 0.047 Can 2, 0.033	9	+	3
	10	+	3
	11	+	3
	12	+	3
Can 1, 0.147 Can 2, 0.087	13	-	-
	14	+	3
	15	+	6
	16	+	4

*Toxin present by mouse inoculation test.

Conclusion: With preirradiation of 1,500,000 rep the sterility dosage appears to lie between F₀ values of 0.087 and 0.329.

TABLE I (Concluded)

Run 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

F ₀	Can No.	Gas Formation	Days to Gas Formation
Noninoculated Controls	1	-	
	2	-	
	3	-	
	4	-	
Inoculated Controls	17	+	2
	18	+	2
	19	+	2
	20	+	2
Can 1, 0.15 Can 2, 0.04	1	+	3
	2	+	3
	3	+	3
	4	+	3
Can 1, 0.27 Can 2, 0.26	5	-	
	6	-	
	7	-	
	8	-	
Can 1, 0.063	9	+	2
	10	+	3
	11	+	2
	12	+	3
Can 1, 0.39 Can 2, 0.25	13	-	
	14	-	
	15	-	
	16	-	

Conclusion: With 1,500,000 rep preirradiation the sterility value lies between F₀ values of 0.063 and 0.27.

SUMMARY OF TABLE I

Run No.	Number of <u>C. botulinum</u> Spores per Can x 10 ⁶	Megarep Preirradiation	F ₀ Required to Sterilize After Irradiation
CB-3	2.30	1.350	less than 0.23
CB-4	5.20	1.350	between 0.11 and 0.21
CB-5	6.30	0.675	more than 0.58
CB-6	6.00	1.000	between 0.41 and 0.80
CB-7	3.80	1.200	between 0.09 and 0.29
CB-8	5.00	0.500	
CB-9	5.00	1.500	more than 0.11
CB-10	6.00	0.500	between 0.77 and 1.06
CB-11	12.00	1.500	between 0.087 and 0.33
CB-12	5.00	1.500	between 0.063 and 0.27
C-1	10.70	0	more than but close to 1.0
C-2	7.20	0	between 0.75 and 1.30
C-3	10.70	0	between 0.36 and 0.93

EFFECT OF CHEMICALS IN THE SUSPENDING MEDIUM ON THE LETHALITY
OF GAMMA RADIATION FOR ANAEROBIC BACTERIAL SPORES

Data presented in Table II and Fig. 2 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 in Progress Report No. 2.

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 III.

Studies have been continued during the past two months to observe the effects of temperature of the suspending medium and the effect of chemicals added to the suspending medium on the lethality of gamma radiation for anaerobic bacterial spores. The work has been confirmatory in nature and so far substantiates the data and results reported in Progress Report No. 2 dated 7 August to 7 October 1955. It is expected that these data presently at hand and data from experiments planned during the next two months will permit publication of this work.

EFFECT OF TEMPERATURES OF THE SUSPENDING MEDIUM DURING IRRADIATION
ON THE LETHALITY OF GAMMA RADIATION FOR ANAEROBIC BACTERIAL SPORES

Table IV and Fig. 3 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. parobotulinum 457 and for PA 3679 spores are shown in Tables V and VI, respectively. This confirms similar data previously presented for these and other anaerobic bacterial spores.

It has been previously reported in Progress Report No. 2 that irradiation-sensitized C. botulinum 213B spores suspended in M/15 phosphate buffer at pH 7.10 must be heated to a minimum critical temperature of 80°C before killing occurs. However, once this temperature is reached, the pre-irradiated spores are killed much more rapidly than are the nonirradiated spores held at the same temperatures. This work has now been extended to PA 3679 spores.

TABLE II

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
1,440,000	88,000	2.465	0.392

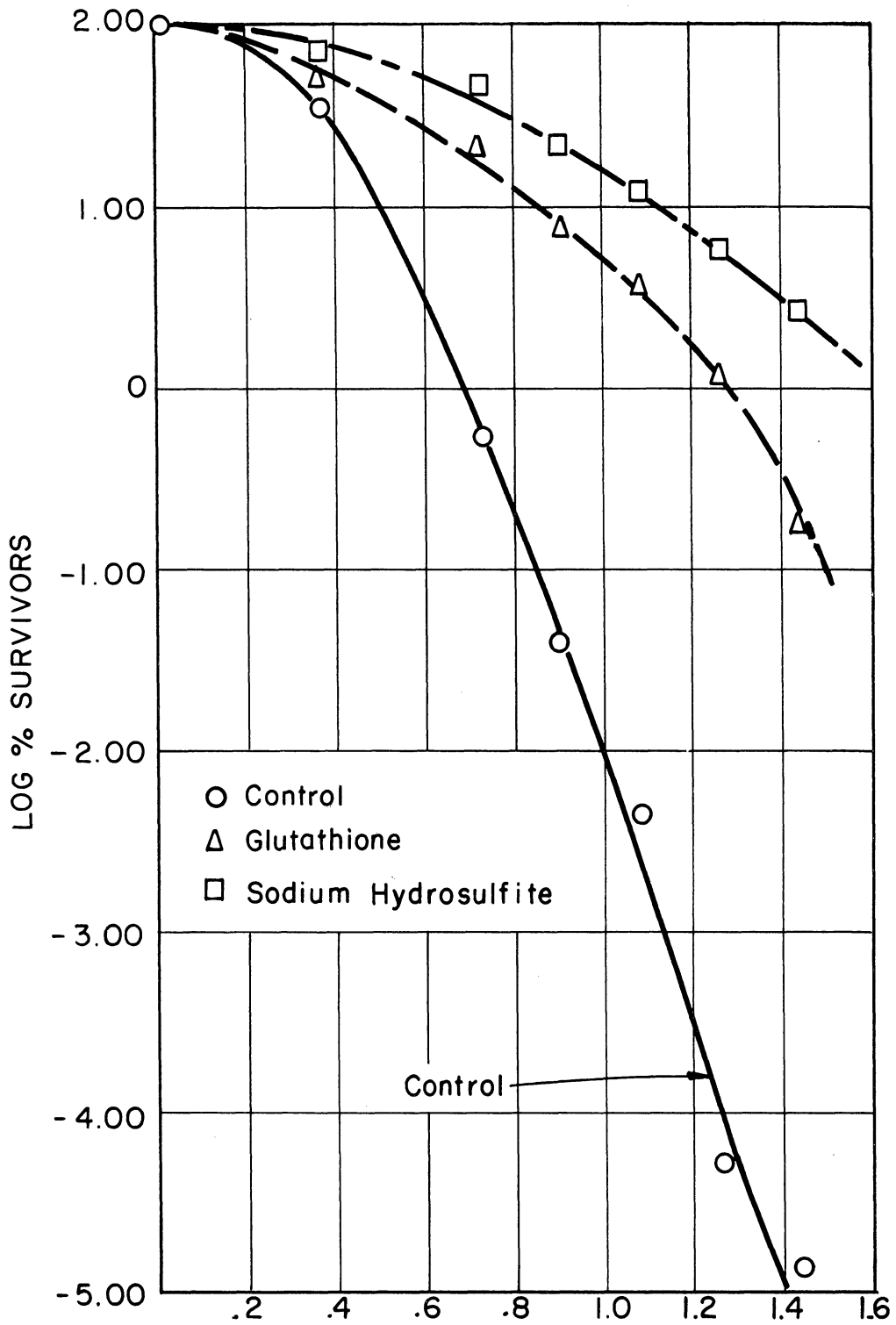


Fig. 2. Effect of protective chemicals at 0.02 M concentration when present in the M/15 phosphate buffer (pH 7.0) used as a suspending medium for PA 3679 spores during irradiation with gamma rays from cobalt-60.

TABLE III

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
<u>pH 3.4</u>			
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
<u>pH 6.75</u>			
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
<u>pH 7.01</u>			
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
<u>pH 8.40</u>			
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
<u>pH 7.17</u>			
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
<u>pH 6.10</u>			
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

TABLE III (Concluded)

Rep	Spores per ml	Percent Survivors	Log Percent Survivors
<u>pH 5.00</u>			
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
<u>pH 4.00</u>			
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
<u>pH 3.23</u>			
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

Data in Table VII and Fig. 4 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 100°C is still necessary before irradiated PA 3679 spores become susceptible to killing with heat.

The data in Table VII also indicate that although the heat resistance of irradiated PA 3679 spores is reduced considerably above 100°C, until this critical temperature range is reached, there is no significant difference between the heat resistances of irradiated and nonirradiated spores.

TABLE IV

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

Dose Rep	Spores per ml	Percent Survivors	Log Percent Survivors
<u>a) Irradiated at 5°C</u>			
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
<u>b) Irradiated at -70°C</u>			
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

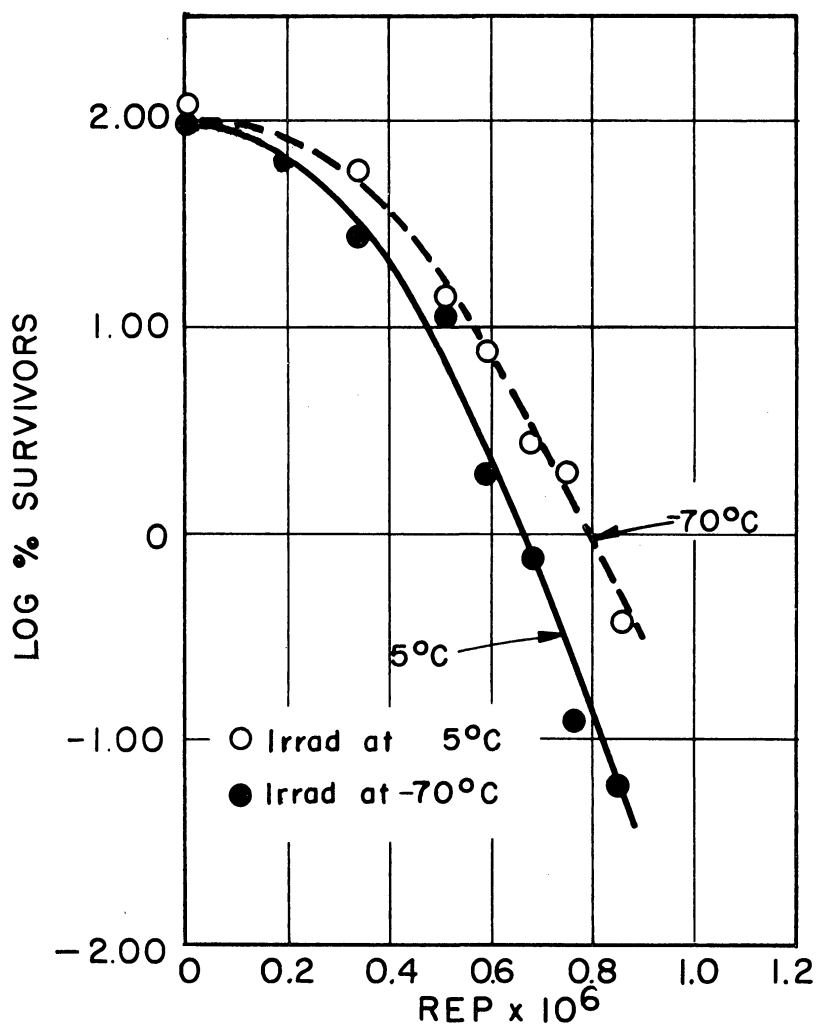


Fig. 3. Effect of temperature during irradiation on the survival of *C. botulinum* 213B spores suspended in M/15 phosphate buffer at pH 7.0.

TABLE V

Effect of Temperatures During Irradiation with Gamma Rays
from Cobalt-60 on the Survival of C. parobotulinum 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

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
<u>a) Irradiated at 5°C</u>			
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
<u>b) Irradiated at -70°C</u>			
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

TABLE VII

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
<u>a) Nonirradiated</u>			
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	-
<u>b) Irradiated with 400,000 rep</u>			
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	-
<u>c) Irradiated with 800,000 rep</u>			
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	-

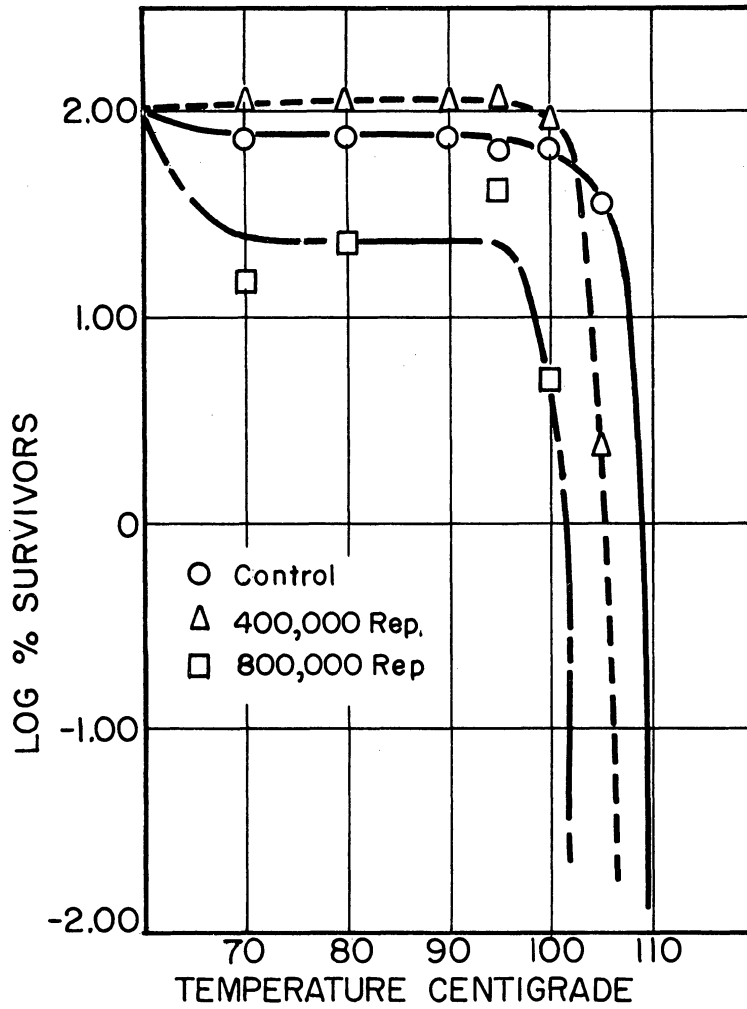


Fig. 4. Effect of postirradiation heating for one hour at various temperatures on previously irradiated PA 3679 spores.

