

CONTRACT RESEARCH PROJECT REPORT

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Title of Contract: Combined Effects of Heat and Radiation
in Food Sterilization

SUMMARY

Irradiated C. botulinum spores were found to be essentially as sensitive to heat after 3 months storage at 4°C in a refrigerator as they were immediately after irradiation.

Irradiated PA 3679 spores are killed quite rapidly by heating at 99°C.

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I. EFFECT OF STORAGE INTERVAL BETWEEN THE TIME OF IRRADIATION AND THE TIME OF PERFORMANCE OF THERMAL-DEATH TIME STUDIES OF THE HEAT RESISTANCE OF IRRADIATED BACTERIAL SPORES

When planning our studies to be carried out in canned meat during 1955-56 and also in response to a letter from Captain Rueben Pomerantz, it was necessary to answer the following question with experimental data: Is the increased heat sensitivity induced in bacterial spores by irradiation affected by storage?

Runs 35, 35A, and 35B were conducted by allowing irradiated C. botulinum 62A spores to stand in the refrigerator overnight before the thermal-death time studies were conducted. The results obtained were normal for C. botulinum spores suspended in 10% gelatin at pH 7.0 and irradiated before heating (Table I).

Runs 36 and 36A were designed to test the effect of a 3-month storage interval at 4°C after irradiation. C. botulinum 213B spores were used in M/15 phosphate buffer for this purpose. The data are presented in Table II and Fig. 1. A comparison with Run 14 (Table III) indicates that storage did not reduce the sensitization effect in this instance, but rather appeared to have accentuated it.

A long-time experiment, designed to test this result further has been set up and will be reported at a later date.

II. EFFECT OF USING UNHEATED BACTERIAL SPORES THAT HAD BEEN HEATED FOR 15 MINUTES AT 85°C WHEN STUDYING THE PROBLEM OF RADIATION SENSITIZATION OF BACTERIAL SPORES TO HEAT

This problem arose as a result of a question in the discussion period following presentation of QMC Paper 544 at the Society of American Bacteriologists Annual Meeting in New York last May.

While it is realized that heating for 15 minutes at 85°C is a very mild treatment and is standard procedure among investigators working with anaerobic bacterial spores, it was considered desirable to check briefly this criticism. Run 39 was conducted for

TABLE I

EFFECT OF PRELIMINARY IRRADIATION BY GAMMA RAYS FROM COBALT-60
ON THE HEAT RESISTANCE OF C. BOTULINUM 62A SPORES

Stored Overnight at 4°C Before Heating at 99°C

Heating Time, min	Equivalent Time at 99°C, min	Control			Irradiated		
		Spore Count per ml	% Survivors	Log % Survivors	Spore Count per ml	% Survivors	Log % Survivors

Suspended in 10% gelatin at pH 7.0

Run 35 (300,000 rep)

0	0	505,000	100.0	2.00	390,000	100.0	2.00
15	10.8	175,000	34.65	1.540	23,000	5.90	0.771
30	24.6	12,500	2.48	0.395	900	0.2305	-0.637
45	38.5	800	0.158	-0.801	40	0.0105	-1.979
60	52.4	lost			3.5	0.000896	-3.047
75	66.3	20	0.00396	-2.402	out		
90	80.0	7	0.001385	-2.858	out		

Run 35A (500,000 rep)

0	0				195,000	100.0	2.00
15	10.8				15,000	7.68	0.886
30	24.6				250	0.128	-0.892
45	38.5		(Same control as Run 35)		15	0.00768	-2.114
60	52.4				1	0.000512	-3.290
75	66.3				out		
90	80.0				out		

Run 35B (900,000 rep)

0	0				85,000	100.0	2.00
15	10.8				770	0.905	-0.043
30	24.6				1	0.00178	-2.749
45	38.5		(Same control as Run 35)				
60	52.4						
75	66.3						
90	80.0						

TABLE II

EFFECT OF STORAGE AT 4°C BETWEEN TIME OF IRRADIATION
AND HEATING ON THE SENSITIVITY TO HEAT
INDUCED BY PREIRRADIATION ON *C. BOTULINUM* 213B SPORES
SUSPENDED IN M/15 PHOSPHATE BUFFER AT pH 7.0

Heating Time, min	Equivalent Time at 99°C, min	Control			Irradiated		
		Spore Count per ml	% Survivors	Log % Survivors	Spore Count per ml	% Survivors	Log % Survivors

Run 36 (450,000 rep) and storage from Jan. 31, 1955,
to May 1, 1955, at 4°C

0	0	1,050,000	100	2.00	130,000	100.0	2.00
5	3.3	690,000	65.7	1.818	110,000	84.5	1.927
10	8.3	460,000	43.8	1.642	27,000	20.8	1.318
15	13.3	290,000	27.6	1.441	8,700	6.69	0.825
20	18.3	350,000	33.3	1.523	2,700	2.08	0.318
30	28.3	150,000	14.3	1.156	250	0.192	-0.716
40	38.3				30	0.023	-1.638

Run 36A (540,000 rep) and storage from Jan. 31, 1955,
to May 1, 1955, at 4°C

0	0				64,000	100.0	2.00
5	3.3				26,000	40.6	1.610
10	8.3				3,500	5.46	0.738
15	13.3	(Same control as Run 36)			1,900	2.97	0.473
20	18.3				320	0.50	-0.301
30	28.3				8	0.125	-1.903
40	38.3				out		

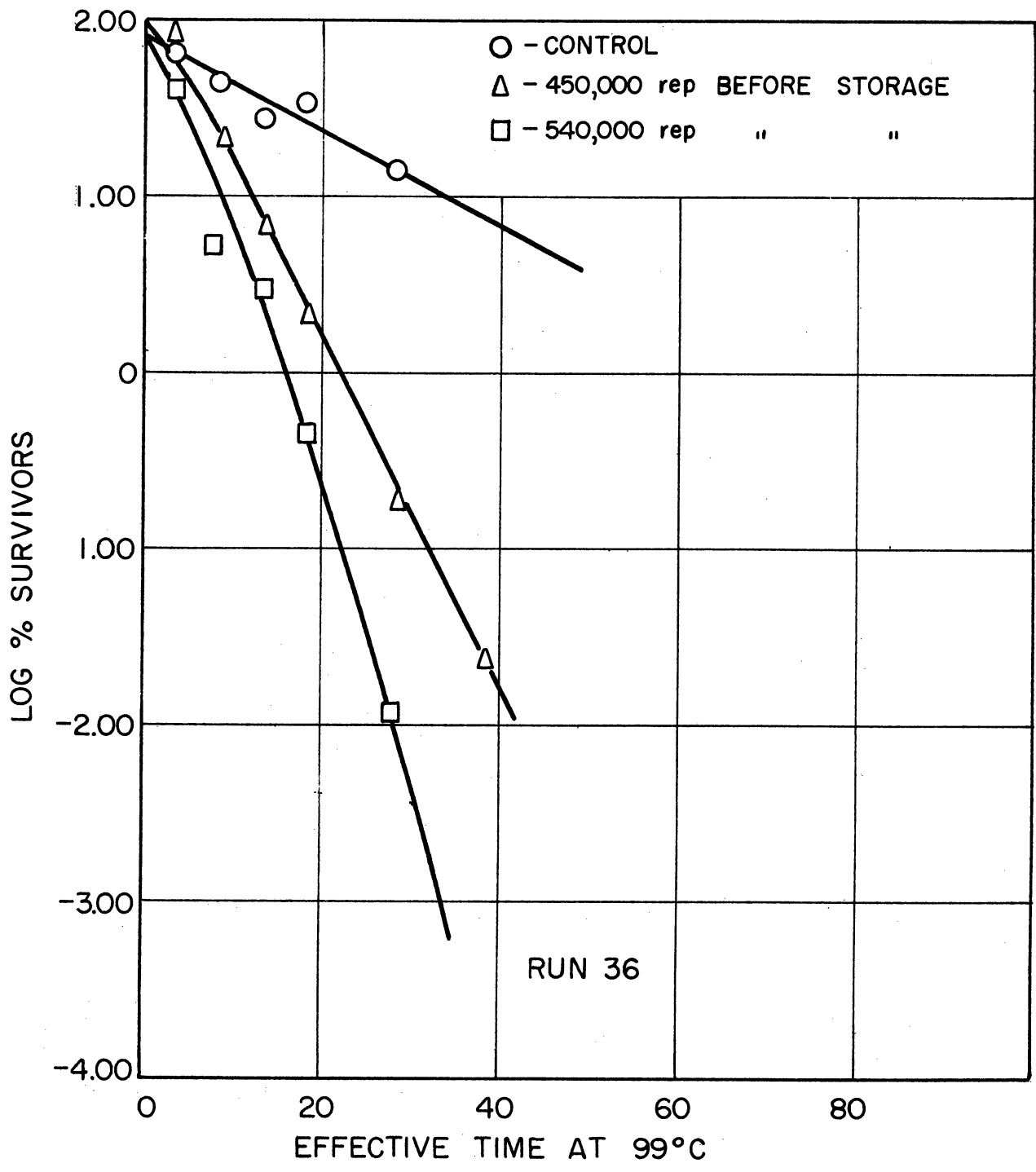


Fig. 1. Effect of storage at 4°C, between the times of irradiation and heating, on the sensitivity to heat induced by preirradiation of *C. botulinum* 213B spores suspended in M/15 phosphate buffer at pH 7.0.

TABLE III

EFFECT OF PRELIMINARY IRRADIATION BY GAMMA RAYS FROM
COBALT-60 ON THE HEAT RESISTANCE OF C. BOTULINUM 213B SPORES

Heating Time, min	Equivalent Time at 99°C, min	Control			Irradiated		
		Spore Count per ml	% Survivors	Log % Survivors	Spore Count per ml	% Survivors	Log % Survivors

A) Suspended in M/15 phosphate buffer at pH 7.0

Run 14 (500,000 rep)

0	0	369,000	100.00	2.000	35,000	100.0	2.0000
15	10.8	106,000	28.70	1.4579	1,400	4.00	0.6021
30	24.6	60,000	16.25	1.2109	240	0.686	-0.1637
45	37.5	25,900	7.03	0.8470	20	0.0572	-1.2426
60	52.4	11,900	3.23	0.5092	2	0.00572	-2.2426
75	66.3	5,100	1.382	0.1405	0	---	---

this purpose. In this experiment previously unheated C. botulinum 213B spores were grown and harvested. They were diluted into M/15 phosphate buffer at pH 7.0 and then four portions were withdrawn. Two of these portions were heated at 85°C for 15 minutes. Then, one portion of the heated and one portion of the unheated samples were set aside as controls and the other two were irradiated at 250,000 rep in the cobalt-60 gamma-ray field. Thermal-death time studies were then made of all four samples. The results are presented in Table IV and Fig. 2. It will be observed that there is no essential difference, for our purposes, between the unheated spores and similar spores that have received a preliminary heat treatment at 85°C for 15 minutes.

TABLE IV

EFFECT OF PREIRRADIATION WITH SUBSEQUENT HEATING AT 99°C
ON UNHEATED C. BOTULINUM 213B SPORES AND ON SIMILAR SPORES THAT
HAD BEEN HEATED AT 85°C FOR 15 MINUTES TO KILL VEGETATIVE CELLS

Heating Time, min	Equivalent Time at 99°C, min	Control			Irradiated		
		Spore Count per ml	% Survivors	Log % Survivors	Spore Count per ml	% Survivors	Log % Survivors

Run 39

		<u>Heated, but not irradiated</u>			<u>Heated and irradiated</u> (250,000 rep)		
0	0	1,260,000	100.0	2.00	1,730,000	100.0	2.00
15	10.8	725,000	57.5	1.76	178,000	10.3	1.013
30	24.6	140,500	11.16	1.048	3,750	0.2165	-0.664
45	38.5	30,500	2.42	0.384	150	0.00865	-2.063
60	52.4	3,200	0.254	-0.595	25	0.001445	-2.837
75	66.3	900	0.0715	-1.346	4	0.000231	-3.636
		<u>Not heated, not irradiated</u>			<u>Not heated but irradiated</u> (250,000 rep)		
0	0	*12,100,000	100.0	2.00	8,300,000	100.0	2.00
5	-	8,550,000	-		5,350,000	64.5	1.81
10		4,450,000	-		1,350,000	16.3	1.212
15	10.8	2,380,000	57.5	1.760	530,000	6.39	0.807
30	24.6	425,000	10.27	1.012	6,050	0.0729	-1.137
45	38.5	83,000	2.00	0.301	1,000	0.0121	-1.917
60	52.4	9,900	0.239	-0.621	175	0.00211	-2.676
75	66.3	2,150	0.052	-1.284	50	0.000603	-3.219

*Contains vegetative cells; a calculation was made indicating 4,140,000 spores were present in this sample at 0 minutes.

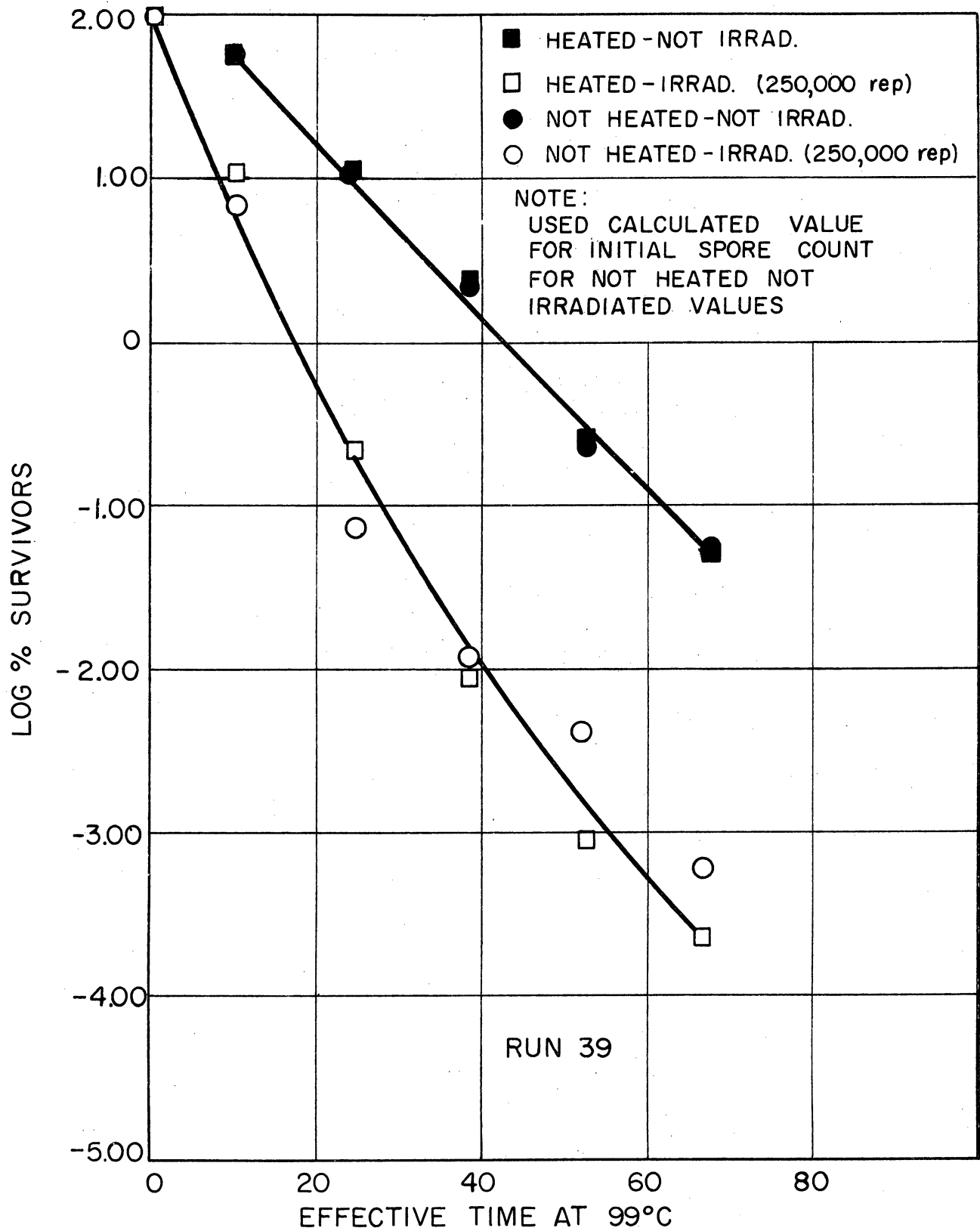


Fig. 2. Effect of preirradiation with 250,000 rep of gamma radiation followed by heating at 99°C on unheated *C. botulinum* 213B spores and on similar spores that had been heated at 85°C for 15 minutes to kill vegetative cells.

III. EFFECT OF TEMPERATURE ON THE SURVIVAL OF BACTERIAL SPORES

A. THE EFFECT OF TEMPERATURE DURING IRRADIATION ON THE SURVIVAL OF BACTERIAL SPORES

This study involves the irradiation of spore suspensions contained in small, heat-sealed glass vials. Irradiation is carried out principally in the center well of the cobalt-60 gamma radiation source. During irradiation the vials are fixed in an especially designed container which permits immersion of the vials in a fluid bath whose temperature can be controlled.

After irradiation, the surviving spores are counted and the numbers found are compared with those surviving in suitable controls. Counting is carried out according to standard techniques similar to those described by Reed, Bohrer, and Cameron.*

B. THE EFFECT OF TEMPERATURE DURING IRRADIATION ON THE SENSITIZA- TION OF BACTERIAL SPORES TO THE SUBSEQUENT LETHAL ACTION OF HEAT

Other workers have suggested that irradiation of food at low temperatures may reduce off-flavor development due to the irradiation treatment. The question naturally arises then as to whether irradiation at low temperatures will still sensitize bacterial spores to the subsequent lethal action of heat. Also, how will high temperatures during irradiation influence this phenomenon? The following experiments have been carried out to answer these questions:

1. PA 3679.—PA 3679 spores were suspended in M/15 phosphate buffer at pH 7.0 and then placed in several glass ampoules which were sealed. The spores were then irradiated at 5 and 95°C, ampoules being withdrawn from the irradiation chamber at specified intervals. Following irradiation, all the vials containing spores were heated at 99°C for 1 hour. An unirradiated control was also included.

Data in Table V and Figs. 3 and 4 indicate that irradiation sensitizes PA 3679 spores in some manner that causes a portion of them to be killed by 1 hour of heating at 99°C. Unirradiated

*J. M. Reed, C. W. Bohrer, and E. J. Cameron, "Spore Destruction Rate Studies on Organisms of Significance in the Processing of Canned Foods," Food Research, 16, 383-408 (1951).

TABLE V

EFFECT OF A COMBINED TREATMENT CONSISTING OF IRRADIATION WITH GAMMA RAYS FROM COBALT-60 FOLLOWED BY HEATING FOR 1 HOUR AT 99°C ON THE SURVIVAL OF PA 3679 SPORES SUSPENDED IN M/15 PHOSPHATE BUFFER AT pH 7.0

Dosage, rep	Spores per ml	% Survivors	Log % Survivors
<u>1a Irradiated at 5°C</u>			
0	2,700,000	100.	2.00
370,000	460,000	17.0	1.230
550,000	95,000	3.51	0.545
650,000	32,000	1.18	0.0719
740,000	14,000	0.519	-0.2848
833,000	3,200	0.118	-0.9281
1,015,000	340	0.0126	-1.8996
1,100,000	54	0.002	-2.699
<u>1b Irradiated at 5°C and Heated for 1 Hour at 99°C</u>			
0	2,300,000	92.0	1.964
370,000	450,000	19.5	1.29
550,000	14,000	0.61	-0.2147
832,000	700	0.0304	-2.5171
1,000,000	2	0.00008	-4.0605
<u>2a Irradiated at 95°C</u>			
0	1,200,000	100.0	2.00
370,000	900,000	75.0	1.875
550,000	570,000	47.5	1.676
740,000	140,000	11.7	1.068
832,000	51,000	4.25	0.628
925,000	30,000	2.5	0.398
1,017,000	10,400	0.866	-0.063
1,100,000	3,100	0.258	-0.588
<u>2b Irradiated at 95°C and heated 1 hour at 99°C</u>			
0	1,100,000	100.0	2.00
370,000	600,000	54.5	1.7364
550,000	120,000	10.9	1.0374
740,000	8,700	0.791	-0.1018
883,000	1,200	0.109	-0.9626
925,000	260	0.0236	-1.6271
1,000,000	36	0.00328	-2.4840
1,100,000	8	0.000726	-3.1391

Control

Held at 95°C for 5 hours (the time required for 925,000 rep); 1,200,000 spores per ml remain, which is the same number as was originally present.

PA 3679 spores show little if any decrease in numbers under like conditions. This finding will be investigated further since it could be significant if a process of irradiation of food followed by heat treatment should be applied in food preservation.

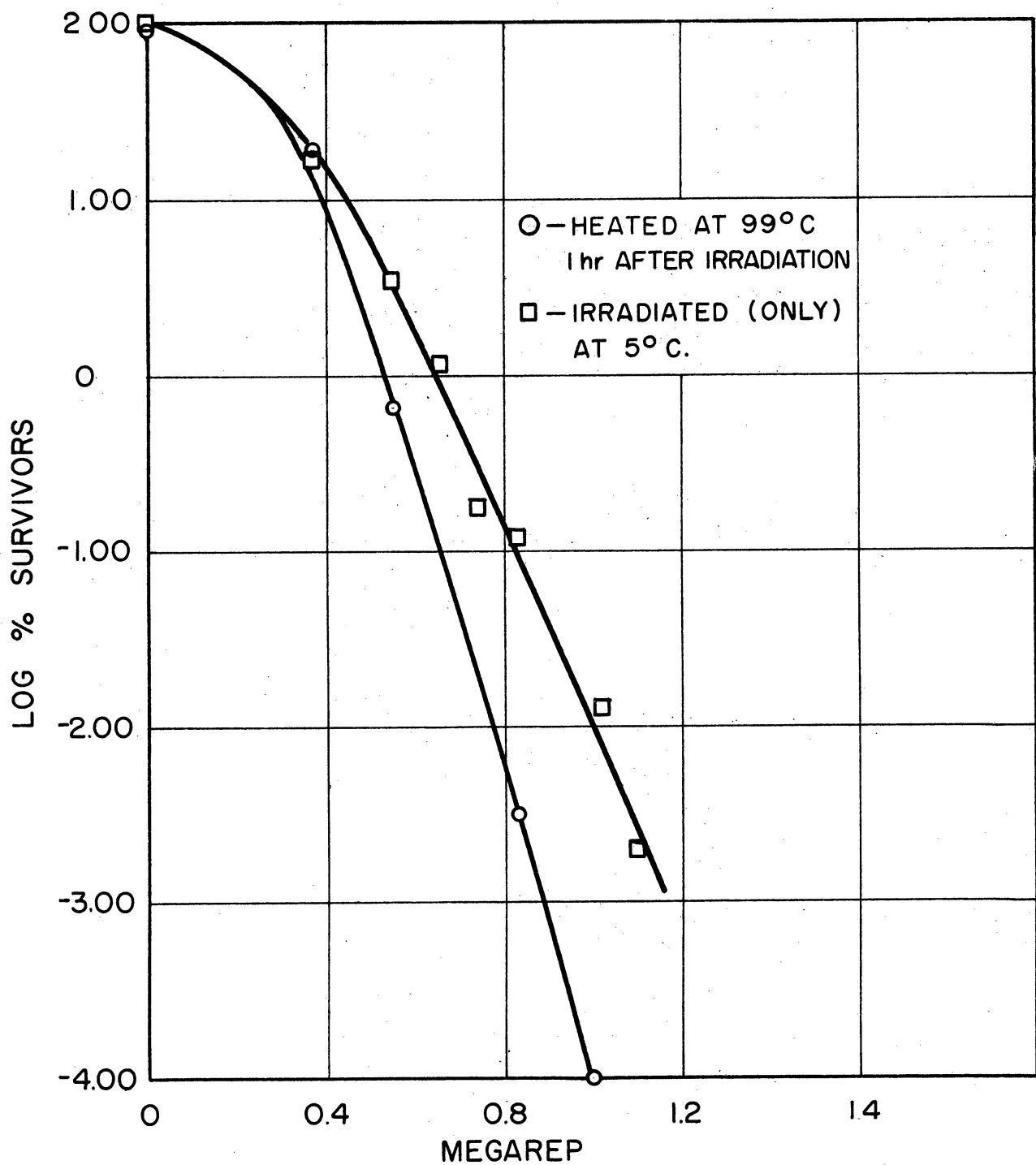


Fig. 3. Effect of heating 1 hour at 99°C on PA 3679 spores that had been previously irradiated with gamma rays from cobalt-60 while held at a temperature of 5°C.

Note: Unboiled control, 2,700,000 spores per ml;
 boiled 1 hour at 99°C, 2,300,000 spores per ml.

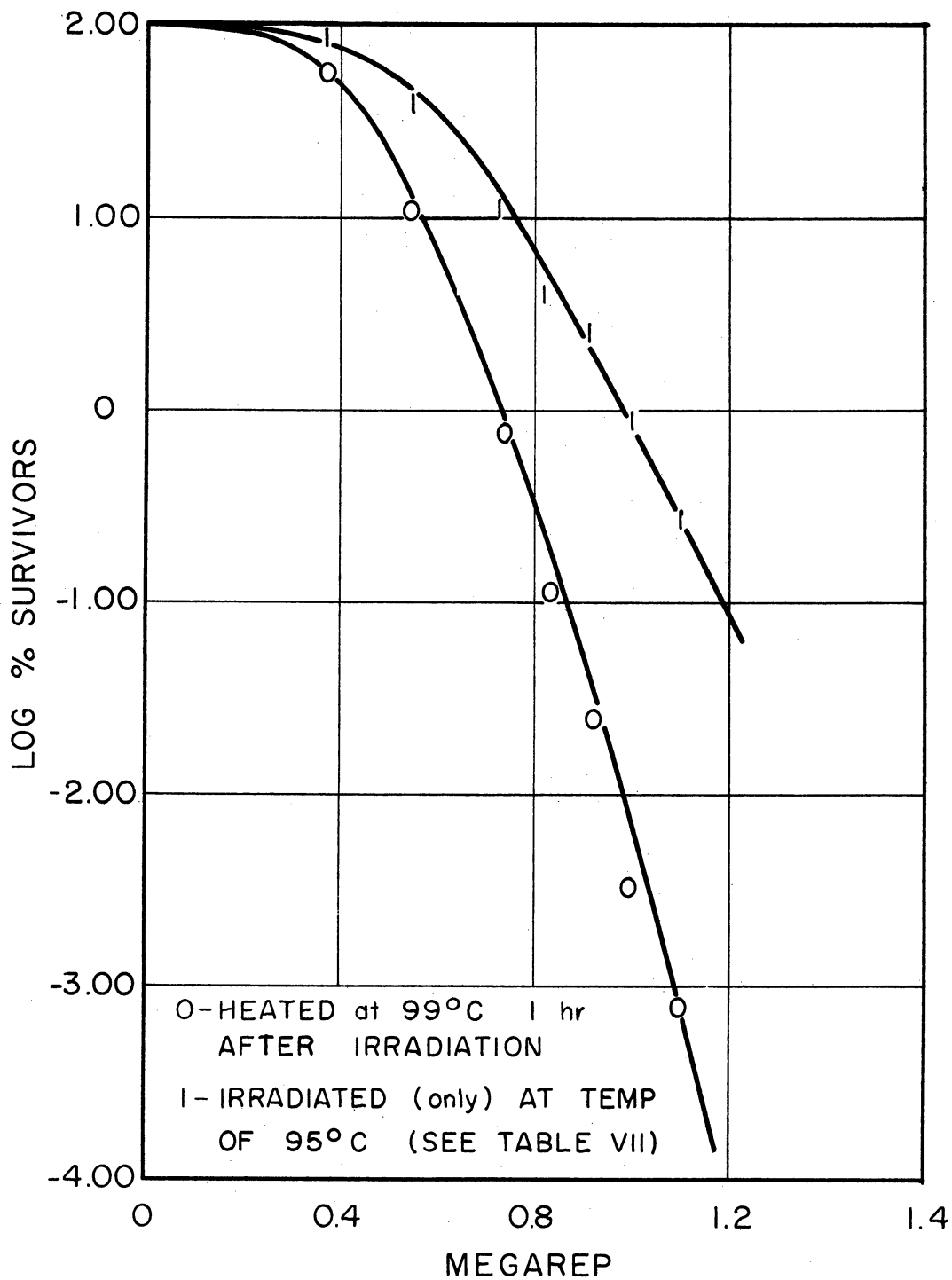


Fig. 4. Effect of heating 1 hour at 99°C on PA 3679 spores that had been previously irradiated with gamma rays from cobalt-60 while held at a temperature of 95°C.

Note: Unboiled control, 2,500,000 spores per ml;
 boiled 1 hour at 99°C, 2,300,000 spores per ml.

