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THE UNIVERSITY OF MICHIGAN
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Final Report

COMBINED EFFECTS OF HEAT AND RADIATION IN FOOD STERILIZATION

7 June 1954 to 7 October 1955

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Project 2272

QUARTERMASTER ACTIVITIES
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OBJECT

The object of this project is to develop an improved food sterilizing process. This work was carried out to investigate the value of a food sterilization technique involving a combined heat and gamma radiation treatment. If the two energy forms were found to be synergistic in their lethal action on spores, it is possible that some combined heat and irradiation process might be equivalent insofar as the effect on microorganisms is concerned but, at the same time, reduce the damage to the food resulting when either form of energy is used alone for sterilization purposes. Specifically then, this project was concerned with determining whether the combined, lethal effect of heat and gamma radiation on bacterial spores is independent or synergistic in nature.

ABSTRACT

Spores of *Clostridium botulinum* strains 62A and 213B as well as those of PA 3679 have been found to be more rapidly killed by heat after irradiation with gamma rays from cobalt-60 than are unirradiated spores. However, heated spores were not sensitized to the subsequent lethal action of radiation. Hence, if a combined heat and radiation treatment is to be used for food sterilization, irradiation should be carried out first. It was found that storage between the time of irradiation and heating did not reduce the sensitization of the spores to heat caused by the irradiation treatment.

When bacterial spores were irradiated at temperatures below those where heat damage occurred it was found that *C. botulinum* 213B spores were more rapidly killed by gamma radiation as the temperature was increased from -70 to 95°C but PA 3679 spores reacted oppositely, being killed more rapidly at temperatures below 58°C than above 80°C.

Studies of the effect of certain chemicals in the medium on the lethality of gamma radiation for bacterial spores showed that many chemicals reduce the lethality of these rays. In particular, reducing agents and sulfur-containing compounds were found to be active in reducing the lethality of gamma radiation for bacterial spores.

CONTRACT RESEARCH PROJECT REPORT

QUARTERMASTER FOOD AND CONTAINER INSTITUTE
FOR THE ARMED FORCES, CHICAGO

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QM Research and Development Center, Natick, Mass.

The University of Michigan
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File No. S-510
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Period: 7 June 1954-
7 October 1955
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Title of Contract: Combined Effects of Heat and Radiation in Food Sterilization

SUMMARY

As set forth in the contractual agreement, studies have been carried out during the past year to determine the following:

1. Whether the combined killing effect of heat and radiation on food spoilage bacteria present in buffer and in foods is independent, additive, detrimental, or synergistic in nature.

It has been found that when *C. botulinum* spores, suspended in phosphate buffer, nutrient broth, or gelatin, are irradiated, they are subsequently more rapidly killed by heating at 99°C than are the unirradiated spores. A similar effect was found for PA 3679 spores in buffer. This indicates that irradiation and heat are synergistic in their lethal action on spores when heating follows irradiation. On the other hand, when the spores were irradiated after heating no difference in the rate of killing by irradiation was observed. This indicates that the two agents are inde-

pendent in their lethal action on spores when irradiation follows heating.

2. Whether bacterial spores react differently to the lethal actions of gamma radiation when irradiated at different temperatures.

This study is not yet complete but at present it can be said that the effect of temperature during irradiation on the lethality of gamma radiation for bacterial spores suggests that C. botulinum and PA 3679 spores are oppositely affected. The former spores are killed more rapidly by gamma radiation as the temperature of irradiation increases but the latter are less rapidly killed at higher temperatures.

3. What effect any process found in (1) above will have on similar spores in meat.

Equipment has been purchased and is being placed in operation to investigate this problem rigorously, since it is the logical application of the positive results reported in (1) above.

4. Whether preliminary irradiation will endow a food with any antiseptic qualities that will increase the effectiveness of a subsequent thermal process.

Studies were carried out in nutrient broth, which is essentially a bouillion soup; in gelatin, which is a food; and in guar gum, which is used as a sausage component. The studies indicate that these food materials do not develop detectable antiseptic qualities when irradiated. In fact, the first two protected the spores to a slight degree during irradiation.

5. Whether chemicals or treatments that affect development of flavor in foods by irradiation alter the lethal effects of irradiation.

This study is necessarily a continuing one. Up to the present, certain amino acids, sulfur containing compounds, and chemicals that are classed as reducing agents, have been shown to protect spores in varying degrees against the lethal action of radiation.

6. Whether or not a correlation exists between heat and radiation resistance of spores.

Work reported by the contractor in Applied Microbiology, Vol. 2, No. 6, p. 330-332, 1954 (but supported by the Michigan Memorial-Phoenix Project) showed that *Clostridium botulinum* spores are more resistant to gamma radiation than are PA 3679 spores. It is common knowledge, on the other hand, that PA 3679 spores are more resistant to heat than *C. botulinum* spores (Food Research, Vol. 19, No. 2, p. 173-181, 1954). So at least for these two spores an inverse correlation exists between heat and radiation when used as sporocidal agents.

7. Whether a biological standard for calibrating the effectiveness of radiation sources can be developed based on a bacterial spore of known radiation sensitivity.

Observations of variations in heat and radiation sensitivity of bacterial spores used in this work demonstrated the need of extensive controls. This indicates that, while a biological standard could possibly be developed based on the killing rate of a known spore preparation, the reliability and general utility of such a test would likely be inferior to other calibration methods now available. This work was therefore abandoned.

8. Some extra experiments were conducted to answer specific questions that arose during the project. Two of these were as follows:

a. Does a time interval between irradiation and heat processing affect the sensitization of spores toward heat developed by irradiation? Preliminary experiments indicate that there is either no difference or a slightly increased sensitivity of irradiated spores to heat after 3 month's storage in a refrigerator. This should permit irradiation of food at one geographical location and shipment to another for heat processing with no decrease in the advantage offered by preirradiation.

b. We used spores that had been preheated at 85°C for 15 minutes to free the suspensions of vegetative cells. Would unheated spores have given the same results? Results of a test experiment show that no differences exist between spores preheated for 15 minutes at 85°C and unheated spores insofar as the purpose of our studies are concerned.

I. CONSECUTIVE TREATMENT OF BACTERIAL SPORES WITH HEAT AND GAMMA RADIATION

A. SUMMARY

Clostridium botulinum spores, strains 62A and 213B, were tested for development of radiation sensitivity subsequent to heating and for heat sensitivity following irradiation. Both strains were tested while suspended in M/15 phosphate buffer at pH 7.0, in nutrient broth at pH 6.7, and in 10% gelatin at pH 7.0. Data presented in tables and graphs show that when these spores were first irradiated with gamma rays from cobalt-60 they were killed much more rapidly by subsequent heat treatment than were similar spores that had not been irradiated. For example, preliminary treatment with 900,000 rep of gamma radiation reduced the subsequent heat resistance of C. botulinum spores approximately fourfold when the spores were suspended in the gelatin medium.

With regard to heat shocking, the data indicate that preliminary heating did not change the rate at which the spores were inactivated by subsequent irradiation. The nature of the medium in which the spores were suspended during irradiation was found to affect the rate at which the spores were killed. Both strains of C. botulinum were killed more slowly in the nutrient broth and gelatin than in the M/15 phosphate buffer.

B. COMBINED EFFECTS OF HEAT AND GAMMA RADIATION IN FOOD STERILIZATION

Ionizing radiations are not presently used for sterilizing foods because undesirable flavor changes and other adverse effects often result at the required dosage levels. Morgan and Reed[1] have shown that preliminary exposure to ionizing radiations lowers the resistance of some bacterial spores to the lethal effects of subsequent heat treatment. However, no work has been presented to indicate the effects of combined heat and radiation treatments on the spores of Clostridium botulinum, which are of critical importance in food sterilization. Therefore, this study has been directed toward determining the effect of preirradiation on the heat resistance of C. botulinum spores, as well as the subsequent irradiation resistance of these spores after heat shocking.

The two strains of *C. botulinum* that were used in this work were obtained from the Hooper Foundation for Medical Research at the University of California. The spore suspensions were prepared according to the procedures of Reed, Bohrer, and Cameron[2] except that Difco bacto-casitone was substituted for casein digest in the growth of medium specified by these workers. Stock spore suspensions were suspended in sterile distilled water and stored at 4°C. The types of toxins produced in the growth media were verified by neutralization tests. Antitoxin used for this purpose was supplied by the New York State Department of Health and the tests were carried out in mice.

Before titering, the stock spore suspensions were heated at 85°C for 15 minutes to kill the vegetative cells. The number of viable spores were then determined by dilution counts, using the Prickett tube technique and pork infusion agar containing 0.1% soluble starch.

For irradiation tests, the spore suspensions were appropriately diluted in M/15 phosphate buffer, nutrient broth, or 10% gelatin. The buffer and gelatin were adjusted to pH 7.0, but the nutrient broth had a pH of 6.7. The solid gelatin kept the spores suspended during irradiation, but both the buffer and broth allowed the spores to settle.

Two series of tests were carried out with both strains of *C. botulinum* in each of the three media. For the first series, aliquot portions of a spore suspension were placed in each of three 18- x 170-mm pyrex glass test tubes. Two of the tubes were immersed in boiling water; the first was removed after 8 minutes and the second after 25 minutes; the third was an unheated control. Following heat treatment, all three of these tubes containing the spore suspensions were irradiated with gamma rays from cobalt-60 at the rate of 170,000 rep per hour. The temperature of irradiation was not controlled and varied from 5 to 17°C as the temperature of the irradiation room changed with the season. At the end of every hour, the irradiation was interrupted for about 10 minutes while samples were pipetted from each tube for spore counting. When the spores were suspended in "solid" gelatin, it was necessary to melt the gelatin by immersing the tube in a 50°C water bath before a sample could be removed. The gelatin was resolidified by plunging the tube into ice water. For tests of the second series, the spore suspensions were placed in test tubes as before. The suspensions were then irradiated in the same way except that no samples were withdrawn. When the irradiation was complete, thermal-death time tests were conducted by placing the tubes in water boiling at 99°C. Samples for counting were pipetted from the tubes and transferred to cold dilution water at the proper time intervals. The number of viable spores were then counted as before. Since the temperatures of the spore suspensions did not rise instantly to 99°C when the test tubes were placed in boiling water, it was necessary to compute the effective heating time at 99°C before a thermal-death time curve could be plotted. A modification of Halvorson's[3] procedure was used for this purpose.

Data presented in Table I and Figs. 1 through 3 show that preirradiation of C. botulinum 62A spores with gamma rays from cobalt-60 sensitized these spores to subsequent heat inactivation. In every case, whether the spores were suspended in phosphate buffer, nutrient broth, or gelatin, they were more rapidly killed by subsequent heating than were the unirradiated spores. Table II and Figs. 4 and 5 show similar data for the spores of C. botulinum 213B. Table III and Fig. 6 show that initial heat shocking of C. botulinum 213B spores suspended in gelatin does not affect the lethal action of subsequent irradiation with gamma rays from cobalt-60. Similar data were obtained for C. botulinum 62A spores in all three suspending media as shown in Table IV.

For purposes of comparison, the F_0 value of the spores was assumed to be that amount of time in minutes at 250°F required to cause the thermal-death time curve to pass through eight logarithmic cycles, and the Z value was taken as 18. On this basis, when C. botulinum 213B spores were suspended in gelatin and then were irradiated with 900,000 rep of gamma rays, their F_0 value was reduced from 1.48 to 0.46. If the lethal effect of the preliminary irradiation with 900,000 rep is included in the overall effect, the F_0 value could be considered to have been still further reduced to 0.35. This represents approximately a fourfold reduction in the F_0 value.

In conclusion, it can be said that preirradiation with gamma rays from cobalt-60 sensitizes the spores of C. botulinum to the subsequent lethal action of heat, but that preliminary heat shocking at 99°C does not affect the lethal action of subsequent gamma irradiation. It was also found that increased amounts of gamma radiation caused increased degrees of sensitization to the subsequent lethal action of heat. This was evidenced by the increasing steepness of the thermal-death time curves developed with those spores that received increasingly higher dosages of gamma radiation. It was also shown that the F_0 value of C. botulinum

TABLE I

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

Heating Time, min	Equivalent Time at 99°C, min	Control: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
A) Suspended in M/15 phosphate buffer at pH 7.05							
Run 2 (170,000 rep) in 16- x 150-mm-OD pyrex test tubes							
0	0	1,980,000	100.0	2.00	1,300,000	100.0	2.000
5	3.3	965,000	48.7	1.688	670,000	51.5	1.712
10	8.3	800,000	40.4	1.606	270,000	20.8	1.318
20	18.3	320,000	16.2	1.210	101,000	7.8	0.892
30	28.3	230,000	11.6	1.065	46,000	3.54	0.549
Run 4 (340,000 rep) in 16- x 150-mm-OD pyrex test tubes							
0	0	1,020,000	100	2.000	440,000	100	2.000
10	8.3	290,000	28.4	1.454	48,000	10.9	1.380
20	18.3	190,000	18.6	1.270	9,500	2.16	0.335
40	38.3	59,000	5.5	0.741	255	0.058	-1.236
60	58.3	13,900	1.36	0.134	4	0.00091	-3.041
80	78.3	3,950	0.387	-0.412	2	0.00045	-3.346
Run 3 (575,000 rep) in 16- x 150-mm-OD pyrex test tubes							
0	0	810,000	100.0	2.000	95,000	100	2.000
10	8.3	310,000	38.3	1.584	12,300	13.0	1.114
20	18.3	250,000	30.9	1.491	1,100	1.16	0.065
40	38.3	49,000	6.1	0.786	10	0.010	-1.979
60	58.3	11,000	1.36	0.134	1	0.001	-2.979
80	78.3	4,500	0.55	-0.259	0	-	-
Run 5 (340,000 rep) in 22- x 175-mm-OD pyrex test tubes							
0	0	1,280,000	100	2.00	440,000	100	2.000
10	6.1				92,500	21.0	1.323
20	15.23	218,000	17.01	1.231	25,570	5.85	0.768
30	24.53				7,050	1.60	0.204
40	33.83	60,600	4.74	0.676	2,850	0.648	-0.198
50	43.20				448	0.102	-0.991
60	52.40	22,750	1.775	0.252	160	0.354	-1.451
70	61.70				56	0.0127	-1.896
80	71.03	7,000	0.546	-0.262	14	0.00318	-2.497

TABLE I (continued)

Heating Time, min	Equivalent Time at 99°C, min	Control: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
B) Suspended in nutrient broth at pH 6.68							
Run 18 (340,000 rep) in 22- x 175-mm-OD pyrex test tubes							
0	0	910,000	100.00	2.0000	640,000	100.00	2.0000
15	10.8	220,000	24.20	1.3838	227,000	35.5	1.550
30	24.6	112,000	12.31	1.0903	43,000	6.72	0.8274
45	37.5	54,500	6.00	0.7782	9,700	1.515	0.1804
60	52.4	19,000	2.09	0.3202	4,850	0.758	-0.1203
75	66.3	12,600	1.385	0.1415	695	0.185	-0.7328
Run 19 (500,000 rep) in 22- x 175-mm-OD pyrex test tubes							
0	0	910,000	100.00	2.0000	410,000	100.0	2.0000
15	10.8	220,000	24.20	1.3838	78,000	19.0	1.2788
30	24.6	112,000	12.31	1.0903	6,000	1.462	0.1650
45	37.5	54,500	6.00	0.7782	775	0.189	-0.7235
60	52.4	19,000	2.09	0.3202	290	0.0705	-1.1518
75	66.3	12,600	1.385	0.1415	30	0.00732	-2.1355
C) Suspended in 10% gelatin at pH 7.0							
Run 31 (300,000 rep)							
0	0	6,870,000	100.0	2.00	4,930,000	100.0	2.00
15	10.8	3,050,000	44.4	1.647	680,000	13.77	1.139
30	24.6	630,000	9.18	0.963	40,000	0.810	-0.091
45	38.5	104,000	1.515	0.181	3,050	0.0618	-1.209
60	52.4	14,000	0.204	-0.690	500	0.0101	-1.995
75	66.3	2,250	0.03275	-1.485	120	0.00243	-2.614
90	80.0	700	0.01020	-1.991	34	0.00068	-3.167
Run 31A (500,000 rep)							
0	0				3,200,000	100.0	2.00
15	10.8				840,000	26.2	1.418
30	24.6				37,000	1.1155	0.048
45	38.5	(Same control as Run 31)			1,650	0.0515	-1.288
60	52.4				155	0.00485	-2.314
75	66.3				20	0.000625	-3.204
90	80.0				5	0.000156	-3.807

TABLE I (continued)

Heating Time, min	Equivalent Time at 99°C, min	Control: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
Run 31B (900,000 rep)							
0	0				550,000	100.0	2.00
15	10.8				9,300	1.69	0.228
30	24.6				15	0.00272	-2.567
45	38.5	(Same control			8	0.00146	-2.835
60	52.4	as Run 31)			0.5		
75	66.3				out		
90	80.0				out		
Run 33 (340,000 rep)							
0	0	1,660,000	100	2.00	1,100,000	100.0	2.00
15	10.8	310,000	18.7	1.272	197,000	17.8	1.251
30	24.6	57,000	3.43	0.536	12,000	1.145	0.059
45	38.5	4,000	0.241	-0.618	375	0.0341	-1.467
60	52.4	350	0.0211	-1.676	45	0.0041	-2.387
75	66.3	135	0.00812	-2.090	1.5	0.000136	-3.866
90	80.0	45	0.00271	-2.567	0.5		
Run 33A (500,000 rep)							
0	0				850,000	100.0	2.00
15	10.8				93,500	11.0	1.042
30	24.6				2,200	0.259	-0.587
45	38.5	(Same control			15	0.00176	-2.754
60	52.4	as Run 33)			out		
75	66.3				out		
90	80.0				out		
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		

TABLE I (concluded)

Heating Time, min	Equivalent Time at 99°C, min	Control: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
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		15	0.00768	-2.114
60	52.4		as Run 35)		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				
60	52.4		as Run 35)				
75	66.3						
90	80.0						

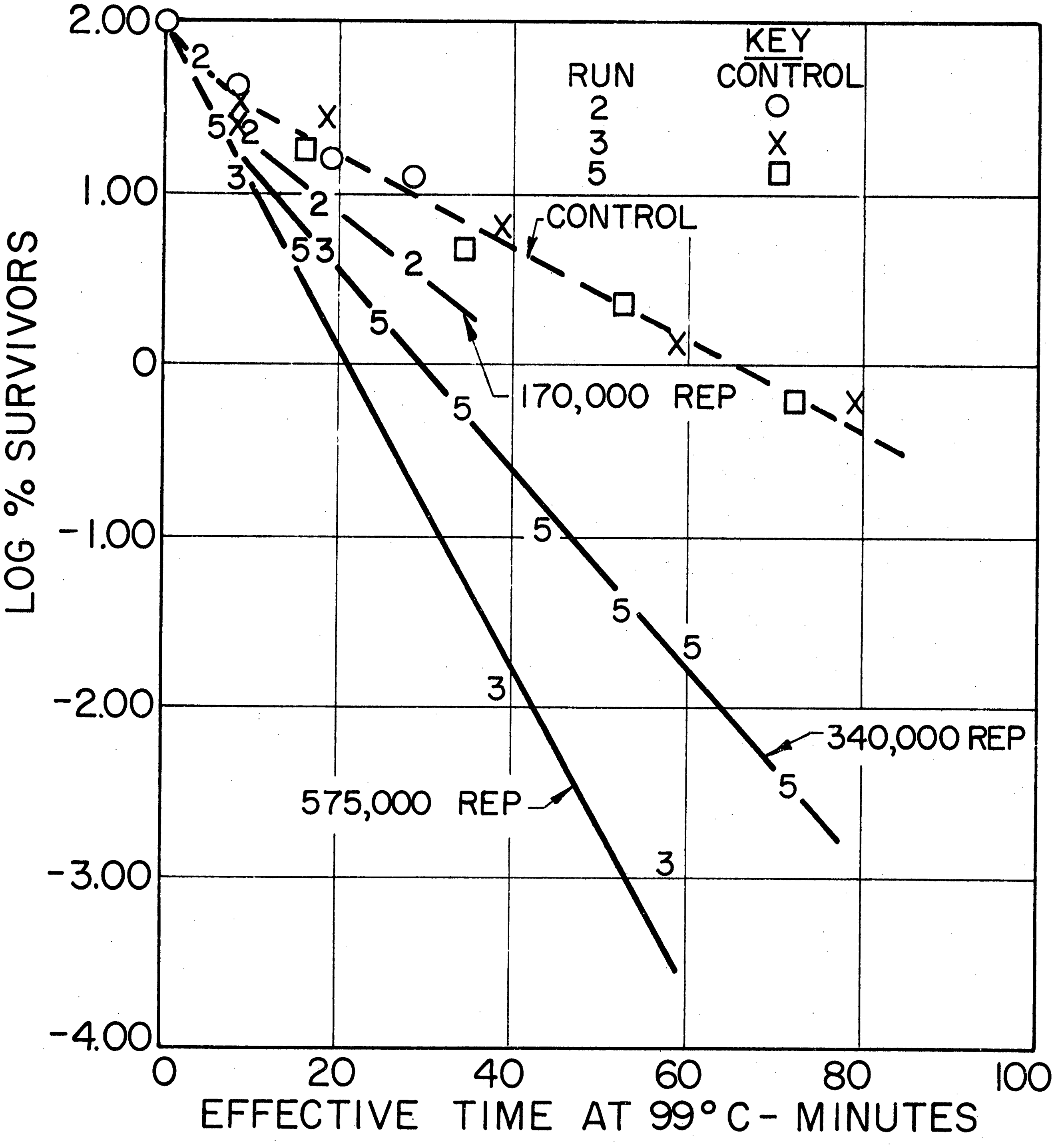

Fig. 1. Effect of preliminary irradiation with gamma rays from cobalt-60 on the heat resistance of C. botulinum 62A spores suspended in M/15 phosphate buffer at pH 7.0.

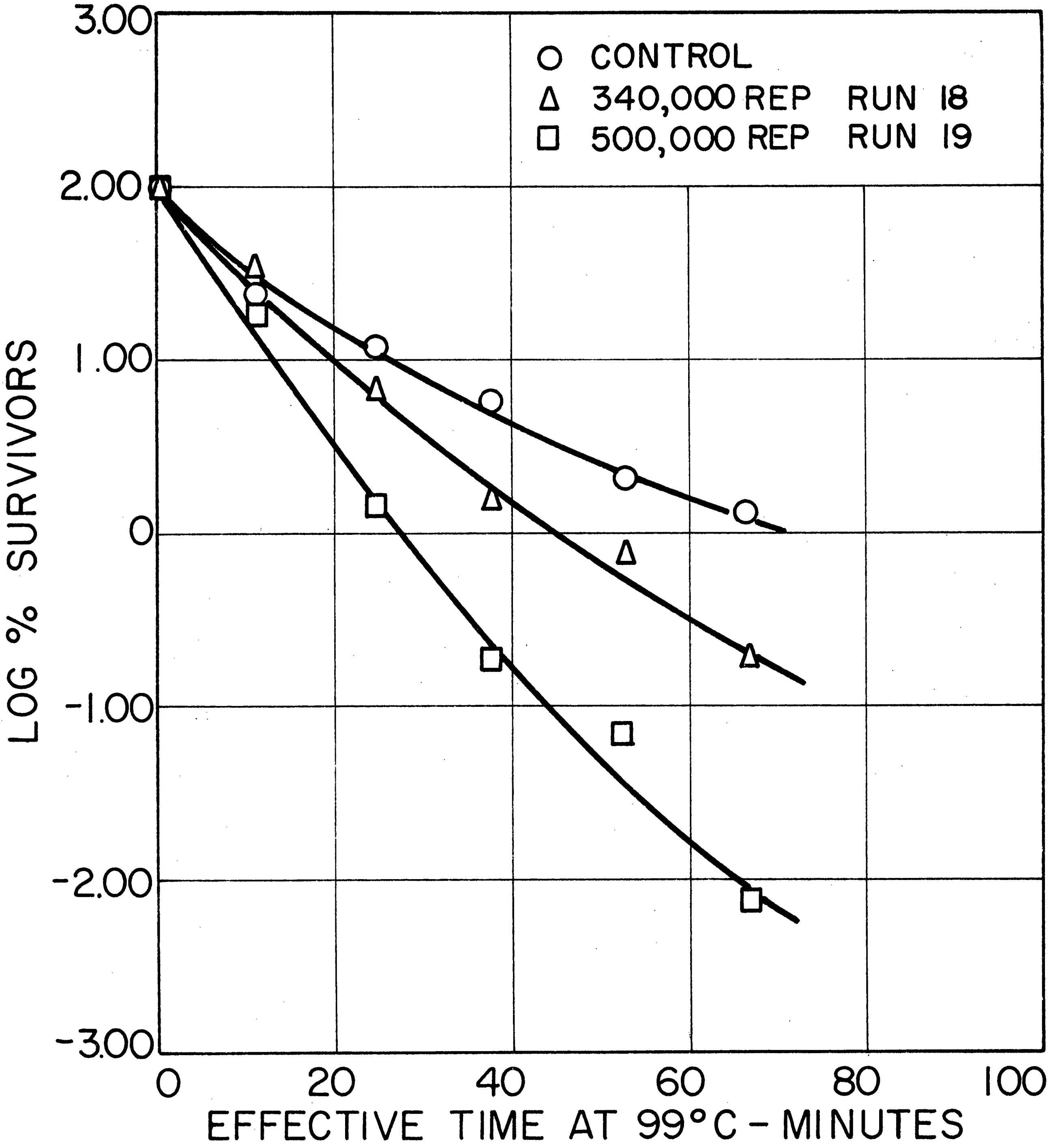

Fig. 2. Effect of preliminary irradiation with gamma rays from cobalt-60 on the subsequent heat resistance of C. botulinum 62A spores suspended in nutrient broth at pH 6.6.

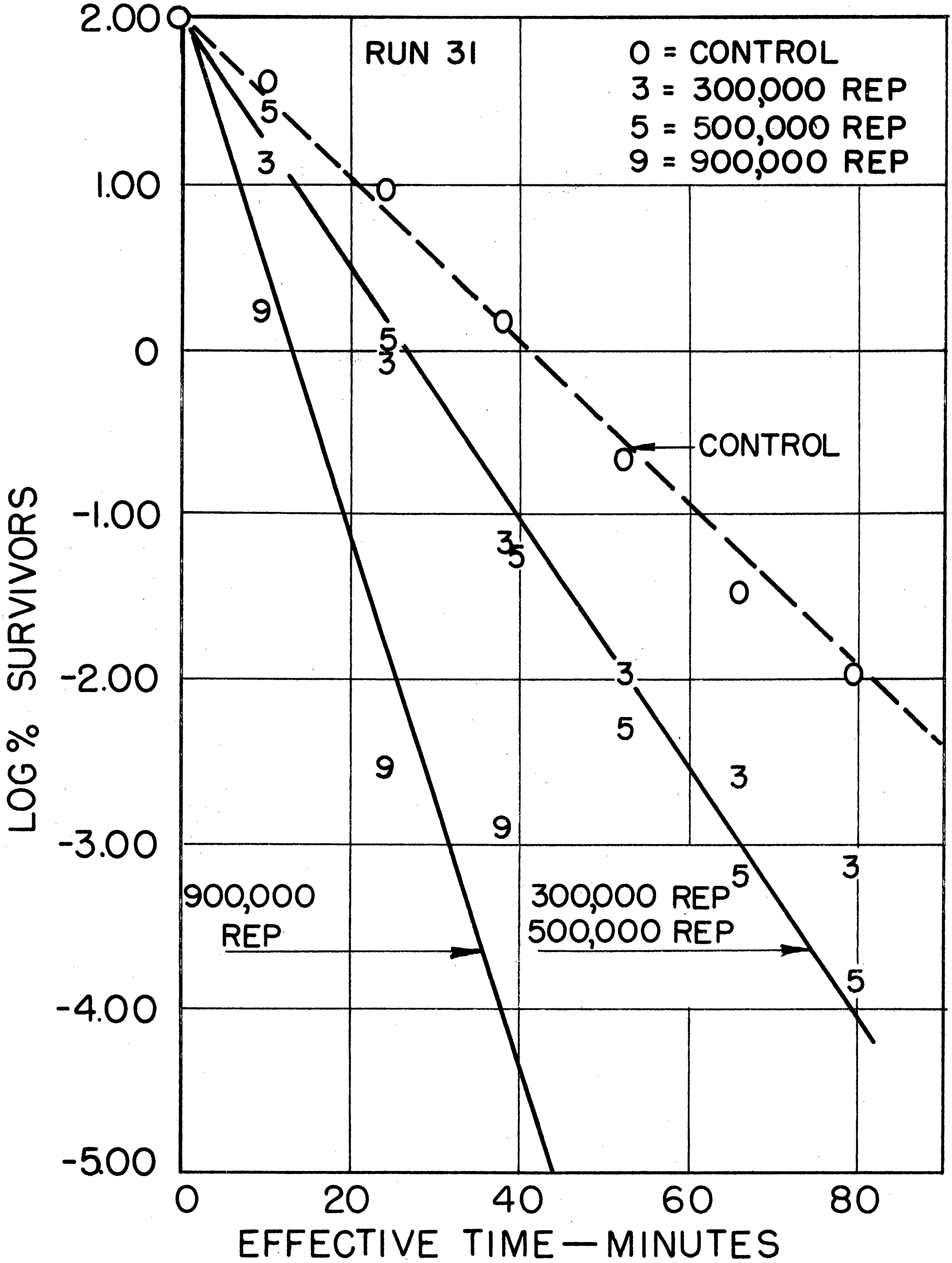

Fig. 3. Effect of preliminary irradiation with gamma rays from cobalt-60 on the subsequent heat resistance of _C. botulinum_ 62A spores suspended in 10% gelatin at pH 7.0.

TABLE II

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 12 (200,000 rep)							
0	0	1,490,000	100	2.00	990,000	100	2.00
10	6.10				375,000	37.8	1.5775
20	15.23	295,000	19.8	1.2706	99,500	10.05	1.0022
30	24.53				43,000	4.34	0.6375
40	33.83	190,000	12.75	1.1055	27,000	2.725	0.4354
50	43.2				10,800	1.091	0.0378
60	52.4	68,000	4.56	0.6590	4,950	0.500	-0.3010
70	61.7				2,450	0.2480	-0.6055
80	71.0	44,500	2.99	0.4757	1,570	0.1589	-0.8089
Run 10 (340,000 rep)							
0	0	2,610,000	100	2.000	1,310,000	100	2.000
10	6.10	705,000	27	1.422	228,000	17.4	1.241
20	15.23	390,000	14.9	1.174	57,000	4.35	0.639
30	24.53	213,000	8.16	0.912	14,300	1.092	0.039
40	33.83	188,000	7.21	0.858	4,400	0.336	-0.473
50	43.2	92,000	3.52	0.547	1,700	0.130	-0.886
60	52.4	70,500	2.70	0.432	615	0.0469	-1.329
70	61.7	48,000	1.84	0.265	134	0.01025	-1.989
80	71.03	53,000	2.02	0.306	125	0.00953	-2.021
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	-	-

TABLE II (continued)

Heating Time, min	Equivalent Time at 99°C, min	Control: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
B) Suspended in nutrient broth* at pH 6.68							
Run 13 (200,000 rep)							
0	0	1,580,000	100	2.000	1,065,000	100	2.000
15	10.8	225,000	14.25	1.1538	100,000	9.40	.9731
30	24.6	67,500	4.28	0.6314	58,000	5.45	.7364
45	37.5	12,600	.798	- .0980	2,400	0.2255	- .6468
60	52.4	9,900	.627	- .2027	235	0.02210	-1.6556
75	66.3	2,490	.1575	- .8027	47	0.00442	-2.3546
Run 11 (340,000 rep)							
0	0	1,450,000	100	2.0000	980,000	100	2.0000
10	6.10	510,000	35.2	1.5465	206,000	21.0	1.3222
20	15.23	230,000	15.86	1.2000	64,000	6.54	0.8156
30	24.53	129,000	8.88	0.9484	20,100	2.05	0.3118
40	33.83	50,000	3.45	0.5378	5,300	0.541	-0.2668
50	43.2	19,000	1.31	0.1173	1,020	0.104	-0.9830
60	52.4	9,800	0.675	-0.1807	103	0.0105	-1.9788
70	61.7	4,900	0.348	-0.4584	18	0.001835	-2.7345
80	71.03	2,900	0.200	-0.6990	6	0.000613	-3.2125
Run 15 (500,000 rep)							
0	0	345,000	100	2.00	129,000	100.0	2.00
15	10.8	65,000	18.8	1.2742	6,600	5.12	0.7093
30	24.6	26,000	7.55	0.8780	1,040	.808	-0.0926
45	37.5	8,350	2.42	0.3838	40	.031	-1.5086
60	52.4	2,880	0.835	-0.0783	2	.0015	-2.8239
75	66.3	775	0.224	-0.6497	0	-	-

*Nutrient broth containing 3 g Difco beef extract, 10 g Difco peptone, 5 g NaCl plus 1000 ml distilled water.

Heating Time, min	Equivalent Time at 99°C, min	Control: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
C) Suspended in 10% gelatin at ph 7.0.							
Run 21A (340,000 rep)							
0	0	1,530,000	100.00	2.00	1,030,000	100.0	2.00
15	10.8	290,000	18.95	1.278	132,000	12.81	1.108
30	24.6	45,000	2.94	0.469	36,500	3.542	0.538
45	38.5	17,500	1.143	0.059	8,000	0.776	-0.110
60	52.4	4,700	0.3075	-0.512	1,940	0.1883	-0.7245
75	66.3	960	0.0627	-1.202	380	0.0369	-1.433
Run 22A (500,000 rep)							
0	0				750,000	100.0	2.00
15	10.8				65,500	8.74	0.842
30	24.6				10,100	1.347	0.129
45	38.5	(Same control as Run 21A)			1,900	0.254	-0.595
60	52.4				340	0.0454	-1.343
75	66.3				30	0.0040	-2.396
Run 27 (340,000 rep)							
0	0	720,000	100.00	2.000	285,000	100.	2.000
15	10.8	217,000	30.20	1.480	79,500	27.9	1.246
30	24.6	79,000	10.97	1.040	24,400	8.56	0.933
45	38.5	18,200	2.53	0.403	8,250	2.89	0.461
60	52.4	6,000	0.834	-0.079	2,230	0.783	-0.106
75	66.3	2,650	0.368	-0.434	1,000	0.351	-0.454
90	80.0	1,090	0.115	-0.939	73	0.0256	-1.592
Run 27A (500,000 rep)							
0	0				295,000	100.0	2.00
15	10.8				63,000	18.55	1.269
30	24.6				9,700	3.285	0.517
45	38.5	(Same control as Run 27)			2,520	0.855	-0.068
60	52.4				550	0.1865	-0.729
75	66.3				62	0.0210	-1.678
90	80.0				17	0.0057	-2.244

TABLE II (continued)

Heating Time, min	Equivalent Time at 99°C, min	Control: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
Run 28 (500,000 rep)							
0	0	1,140,000	100.0	2.00	643,000	100.0	2.00
15	10.8	455,000	39.9	1.601	124,000	19.28	1.285
30	24.6	168,000	14.75	1.169	24,400	3.79	0.579
45	38.5	74,500	6.54	0.815	5,400	0.839	-0.076
60	52.4	22,100	1.94	0.288	1,400	0.218	-0.661
75	66.3	9,550	0.838	-0.076	160	0.0249	-1.603
90	80.0	2,450	0.215	-0.667	19	0.00285	-2.545
Run 28A (900,000 rep)							
0	0				124,000	100.0	2.00
15	10.8				4,200	3.39	0.530
30	24.6				360	0.291	-0.536
45	38.5	(Same control			7	0.00565	-2.248
60	52.4	as Run 28)			0.5	0.00040	-3.398
75	66.3				out		
90	80.0				out		
Run 30 (500,000 rep)							
0	0	5,300,000	100.0	2.000	4,300,000	100.0	2.00
15	10.8	3,100,000	58.5	1.767	980,000	22.8	1.358
30	24.6	795,000	15.0	1.176	370,000	8.60	0.935
45	38.5	470,000	8.87	0.948	120,000	2.79	0.446
60	52.4	159,000	3.00	0.478	44,500	1.033	0.015
75	66.3	76,000	1.432	0.157	6,700	0.1556	-0.808
90	80.0	18,300	0.345	-0.462	1,540	0.0358	-1.446
Run 30A (900,000 rep)							
0	0				580,000	100.0	2.00
15	10.8				104,000	17.93	1.254
30	24.6				11,500	1.982	0.298
45	38.5	(Same control			2,280	0.393	-0.445
60	52.4	as Run 30)			235	0.0405	-1.392
75	66.3				24	0.00414	-2.383
90	80.0				2.5	0.000432	-3.364

TABLE II (concluded)

Heating Time, min	Equivalent Time at 99°C, min	Control: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
Run 34 (500,000 rep)							
0	0	10,300,000	100.0	2.00	5,030,000	100.0	2.00
15	10.8	3,100,000	30.1	1.479	625,000	12.4	1.094
30	24.6	480,000	4.66	0.669	133,000	2.64	0.422
45	38.5	145,000	1.41	0.149	18,600	0.370	-0.431
60	52.4	73,500	0.714	-0.146	1,900	0.0378	-1.422
75	66.3	25,500	0.2285	-0.640	260	0.00517	-2.286
90	80.0	4,500	0.0438	-1.358	18	0.000358	-3.446
Run 34A (700,000 rep)							
0	0				910,000	100.0	2.00
15	10.8				47,000	5.17	0.724
30	24.6				1,260	0.139	-0.857
45	38.5	(Same control as Run 34)			15	0.00165	-2.782
60	52.4				out		
75	66.3				out		
90	80.0				out		

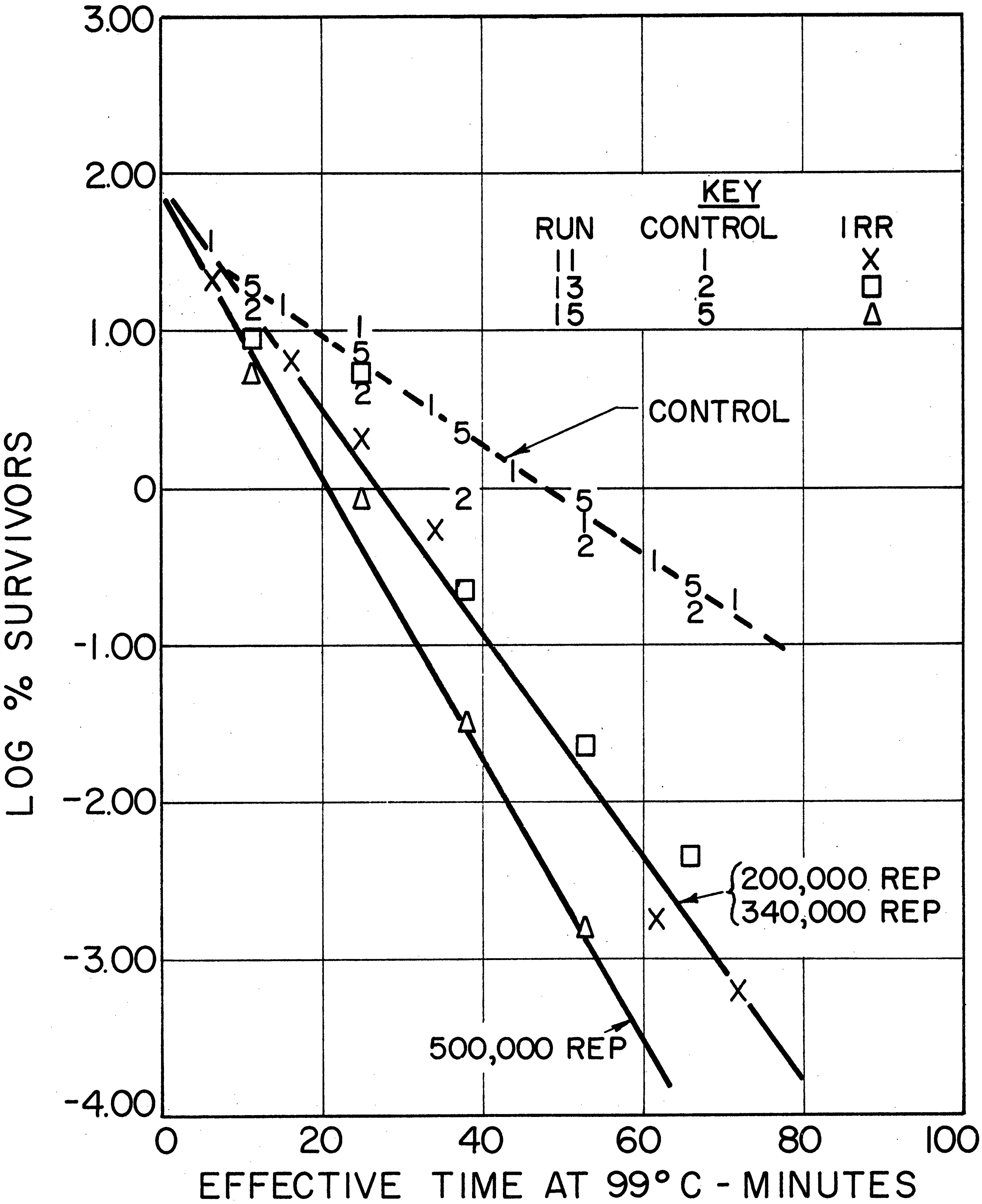

Fig. 4. Effect of preliminary irradiation with gamma rays from cobalt-60 on the subsequent heat resistance of C. botulinum 213B spores suspended in nutrient broth at pH 6.7.

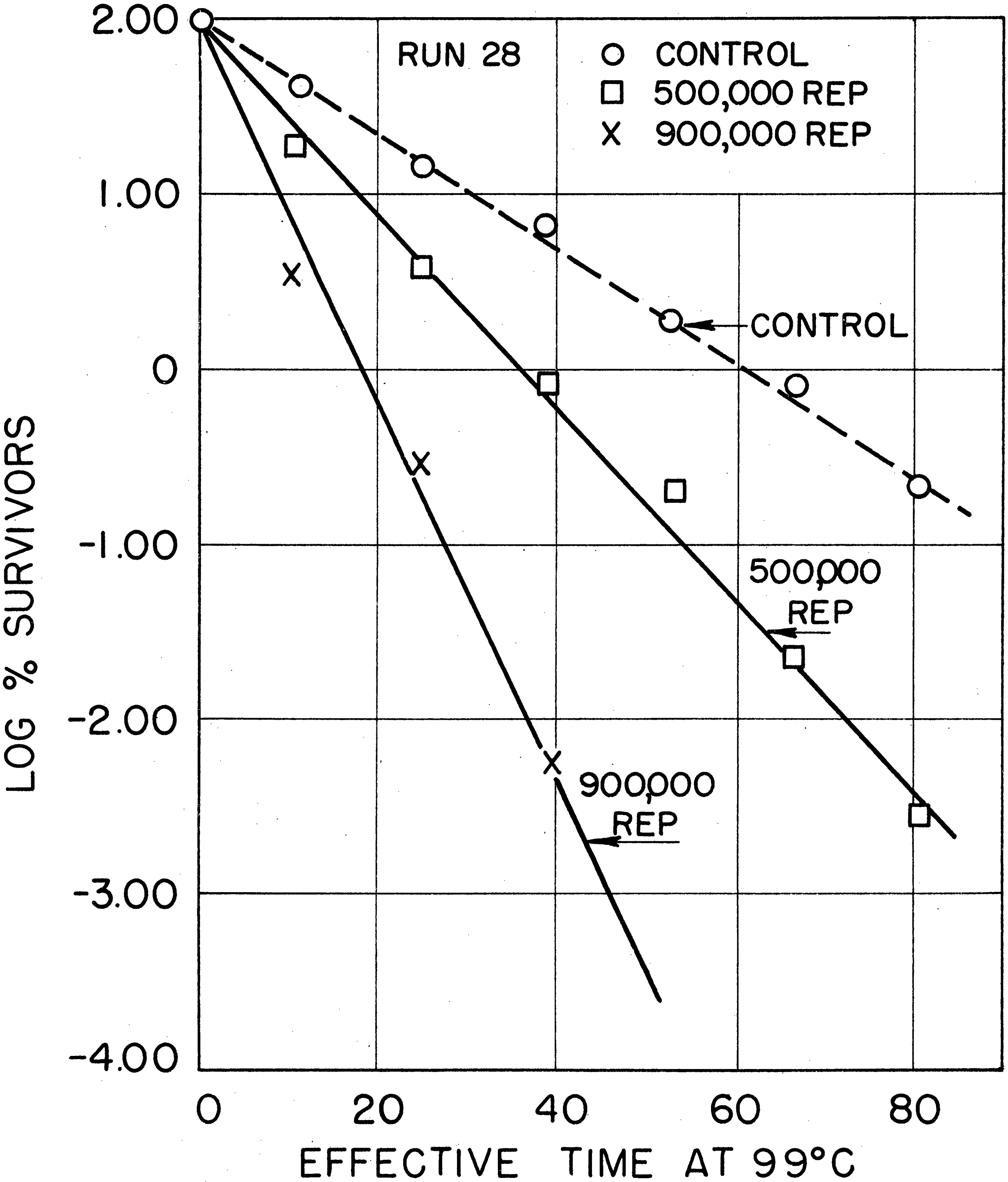

Fig. 5. Effect of preliminary irradiation with gamma rays from cobalt-60 on the heat resistance of C. botulinum 213B spores suspended in 10% gelatin at pH 7.0.

TABLE III

EFFECT OF PRELIMINARY HEAT TREATMENT ON IRRADIATION RESISTANCE OF *C. BOTULINUM* 62A SPORES TO GAMMA RAYS FROM COBALT-60

Irradiation dosage, rep	Unheated Count	% Survivors	Log % Survivors	Boiled Count	% Survivors	Log % Survivors	Boiled Count	% Survivors	Log % Survivors	Remarks
A) Suspended in M/15 phosphate buffer at pH 7.05 in 22- x 175-mm-OD pyrex test tubes										
Run 6				9 min[1]			20 min[2]			
0	1,620,000	100	2.000	630,000	100	2.000	430,000	100	2.000	[1]5.2 min equivalent time at 99°C
170,000	1,480,000	91.2	1.961	560,000	89.0	1.950	384,000	89.3	1.951	
340,000	810,000	50.0	1.699	460,000	73.0	1.864	258,000	60.0	1.778	
510,000	460,000	28.4	1.454	230,000	36.5	1.563	197,000	45.8	1.661	[2]15.2 min equivalent time at 99°C
680,000	114,000	7.03	0.847	70,000	11.1	1.046	72,000	16.7	1.223	
Run 7				8 min[3]			25 min[4]			[3]4.4 min equivalent time at 99°C
0	133,000	100	2.00	20,000	100	2.00	4,500	100	2.00	
170,000	64,500	48.5	1.686	15,000	75	1.875	6,500	144	2.158	
340,000	33,000	24.8	1.395	14,000	70	1.846	800	17.5	1.243	[4]20 min equivalent time at 99°C
510,000	7,500	5.64	0.751	2,500	12.5	1.097	330	7.3	0.864	
680,000	550	0.414	-0.383	350	1.75	0.244	81	1.8	0.256	

TABLE III (concluded)

Irradiation dosage, rep	Unheated Count	% Survivors	Log % Survivors	Boiled Count	% Survivors	Log % Survivors	Boiled Count	% Survivors	Log % Survivors	Remarks
Run 8				8 min[3]			25 min[4]			
0	430,000	100	2.000	74,000	100	2.00	7,000	100	2.000	[1]5.2 min equivalent time at 99°C
170,000	490,000	114	2.057	54,500	73.6	1.868	5,400	77.2	1.888	
340,000	193,000	44.8	1.652	43,000	58.1	1.765	4,300	61.5	1.789	
510,000	41,000	9.55	0.980	13,600	18.4	1.265	3,000	42.8	1.632	[2]15.2 min equivalent time at 99°C
680,000	8,100	1.88	0.274	3,600	4.86	0.688	515	7.36	0.878	
850,000	900	0.209	-0.680	537	0.725	-0.14	40	0.572	-0.242	
1,020,000	45	0.0109	-1.962	18	0.243	-1.614	3	0.0428	-1.368	[3]4.4 min equivalent time at 99°C
B) Suspended in nutrient broth at pH 6.68										
Run 20				8 min[1]			25 min[2]			[4]20 min equivalent time at 99°C
0	910,000	100.0	2.0000	415,000	100.0	2.00	620,000	100.0	2.000	
170,000	910,000	100.0	2.0000	364,000	87.8	1.944	475,000	76.6	1.885	
340,000	770,000	84.6	1.928	310,000	74.7	1.874	315,000	50.8	1.706	
510,000	350,000	38.5	1.586	141,000	34.2	1.534	203,000	32.7	1.515	
680,000	105,000	11.54	1.063	91,500	22.05	1.344	57,000	9.19	0.963	
850,000	23,700	2.60	0.416	25,200	6.07	0.784	16,300	2.63	0.420	
1,020,000	5,200	0.572	-0.242	8,250	1.985	0.298	3,900	0.629	-0.202	
C) Suspended in 10% gelatin at pH 7.0										
Run 32				8 min			25 min			
0	720,000	100	2.000	570,000	100	2.00	183,000	100.0	2.00	
340,000	590,000	82.0	1.914	385,000	67.6	1.83	154,000	84.0	1.924	
680,000	169,000	23.5	1.371	160,000	28.1	1.449	73,000	39.9	1.601	
850,000	55,000	7.64	0.883	101,000	17.7	1.248	27,500	15.02	1.177	
1,020,000	13,100	1.82	0.260	26,000	4.56	0.660	7,250	3.96	0.598	
1,230,000	2,900	0.403	-0.394	4,000	0.702	-0.153	1,020	0.556	-0.254	
1,360,000	1,200	0.1667	-0.778	1,100	0.193	-0.714	315	0.172	-0.764	

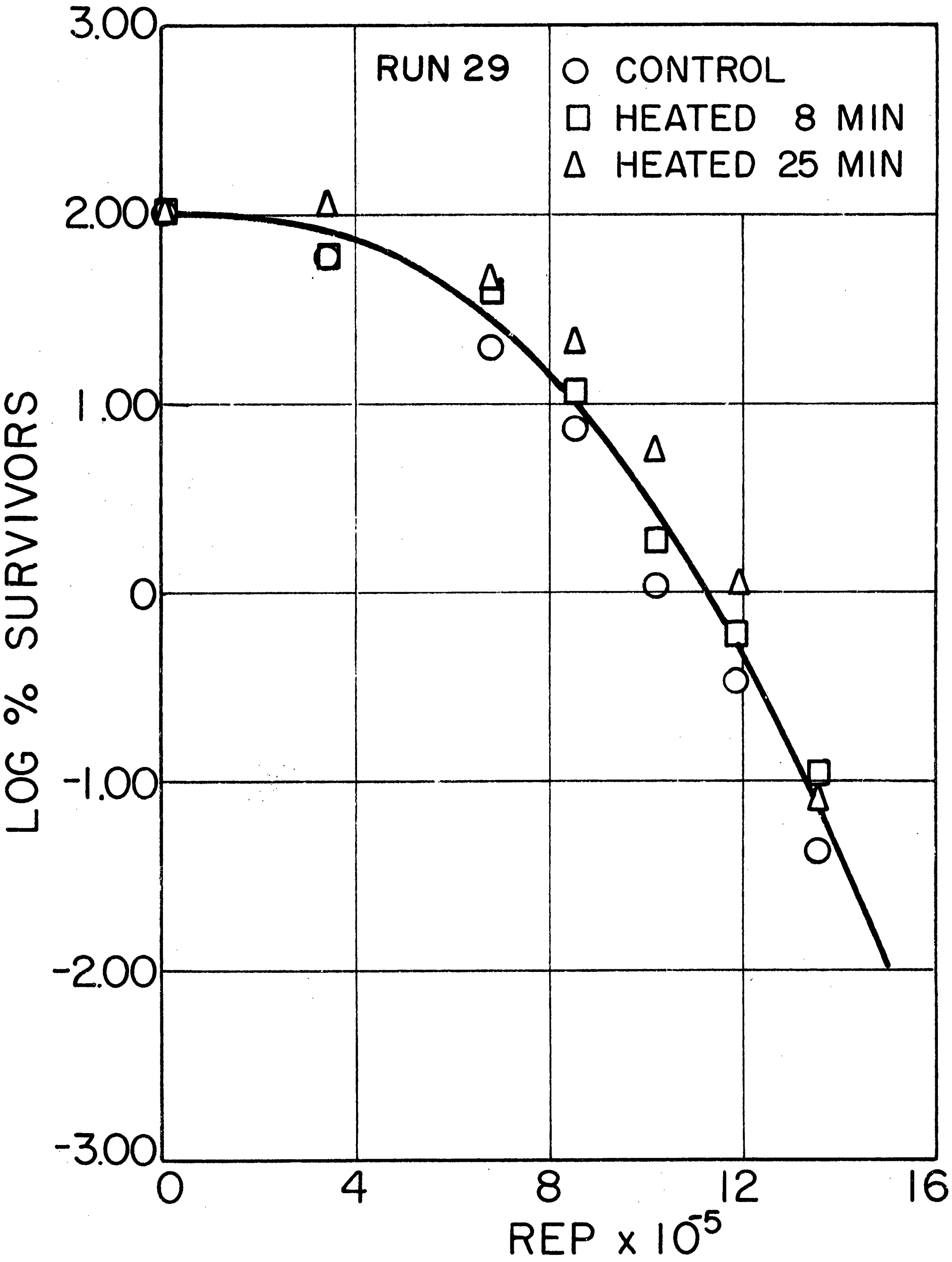

Fig. 6. Effect of preliminary heating at 99°C on the subsequent radiation resistance of C. botulinum 213B spores suspended in 10% gelatin at pH 7.0.

TABLE IV

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

Irradiation dosage, rep	Unheated Count	% Survivors	Log % Survivors	Boiled Count	% Survivors	Log % Survivors	Boiled Count	% Survivors	Log % Survivors	Remarks
A) Suspended in phosphate buffer at pH 7.0										
Run 16				8 min[1]			25 min[2]			
0	1,290,000	100	2.000	750,000	100.0	2.000	267,000	100.0	2.000	[1]4.4 min equivalent time at 99°C
170,000	1,100,000	85.3	1.9310	520,000	69.3	1.8407	249,000	93.3	1.9699	
340,000	690,000	53.5	1.7284	354,000	47.2	1.6739	168,500	63.1	1.8000	
510,000	333,000	25.8	1.4116	162,000	21.6	1.3345	98,500	36.85	1.5664	[2]20 min equivalent time at 99°C
680,000	70,000	5.43	0.7348	46,000	6.13	0.7875	21,000	7.87	0.8960	
850,000	10,100	0.783	-0.1062	9,500	1.266	0.1024	3,590	1.345	0.1287	
1,020,000	930	0.0721	-1.1421	1,140	0.152	-0.8182	520	.1945	-0.7111	
B) Suspended in nutrient broth at pH 6.68										
Run 17										
0	1,580,000	100.0	2.0000	835,000	100.0	2.0000	310,000	100.0	2.0000	
170,000	1,460,000	97.5	1.9890	905,000	108.3	2.0346	275,000	88.7	1.9479	
340,000	995,000	63.0	1.7993	545,000	65.3	1.8149	180,000	58.1	1.7642	
510,000	820,000	51.9	1.7152	385,000	46.2	1.6646	165,000	53.25	1.7263	
680,000	285,000	18.03	1.2560	177,000	21.2	1.3263	83,000	26.8	1.4281	
850,000	98,500	6.25	0.7959	84,500	10.12	1.0052	48,000	15.5	1.1903	
1,020,000	17,600	1.113	0.0465	21,600	2.59	0.4133	16,100	5.2	0.7160	

TABLE IV (concluded)

Irradiation dosage, rep	Unheated Count	% Survivors	Log % Survivors	Boiled Count	% Survivors	Log % Survivors	Boiled Count	% Survivors	Log % Survivors	Remarks
C) Suspended in 10% gelatin.										
Run 23				8 min			25 min			
0	1,580,000	100.0	2.00	560,000	100	2.00	1,230,000	100.0	2.00	
170,000	1,310,000	82.9	1.919	520,000	92.9	1.968	104,000	84.5	1.927	
340,000	1,240,000	78.5	1.895	280,000	50.0	1.699	74,000	60.1	1.780	
510,000	720,000	45.6	1.659	290,000	51.7	1.714	74,000	60.1	1.780	
680,000	350,000	22.15	1.345	150,000	26.8	1.428	40,000	32.5	1.512	
850,000	230,000	14.56	1.1163	73,000	13.02	1.115	22,000	17.9	1.253	
Run 29				8 min			25 min			
0	1,100,000	100.0	2.000	480,000	100.0	2.00	184,000	100.0	2.00	
340,000	635,000	57.7	1.762	255,000	53.1	1.726	200,000	108.8	2.037	
680,000	210,000	19.1	1.281	186,000	38.75	1.588	77,000	41.8	1.612	
850,000	81,000	7.36	0.868	54,500	11.34	1.055	33,500	18.2	1.260	
1,020,000	14,700	1.336	0.1126	16,700	3.48	0.542	10,300	5.59	0.747	
1,190,000	3,600	0.3275	-0.485	3,000	0.625	-0.204	2,060	1.1118	0.048	
1,360,000	450	0.0409	-1.388	550	0.1144	-0.941	155	0.0842	-1.085	

spores could be reduced approximately fourfold by preirradiation. The amount of this effect was found to be less in gelatin and nutrient broth than in phosphate buffer.

II. SPECIAL EXPERIMENTS

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

When planning our experiments soon to be carried out in meat and also in response to a letter from Captain Reuben 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? Three lines of attack on the problem were used.

1. Runs 35, 35A, and 35B were conducted by allowing the irradiated *C. botulinum* 62A spores to stand in the refrigerator overnight before the thermal-death time studies were conducted. The data obtained were normal for *C. botulinum* 62A spores suspended in 10% gelatin at pH 7.0 and irradiated before heating (Table 1C).

2. 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 V and Fig. 7. A comparison with Run 14 (Table IIA) indicates that storage did not reduce the sensitization effect in this instance, but rather appeared to have accentuated it.

3. A long-time experiment has been set up and will be reported on at a later date.

B. EFFECT OF USING UNHEATED BACTERIAL SPORES IN PLACE OF 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 in May.

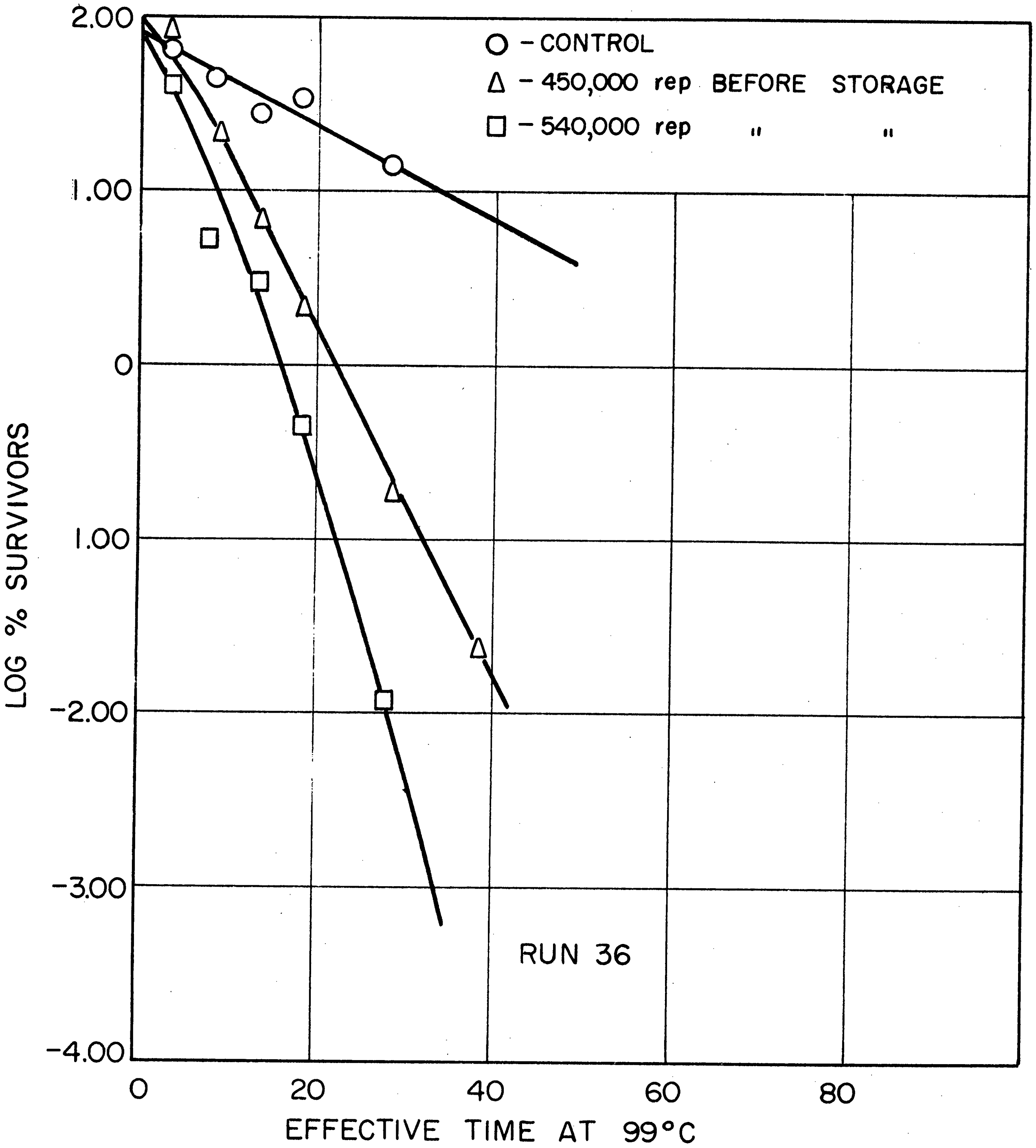

Fig. 7. Effect of 3 month's storage at 4°C between the times of irradiation and heating on the sensitivity to heat induced by pre-irradiation of C. botulinum 213B spores suspended in M/15 phosphate buffer at pH 7.0.

TABLE V

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: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: 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	(Same control as Run 36)			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				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		

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 37 was conducted for this purpose. In this experiment, previously preheated 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 VI and Fig. 8. It will be observed that there is no essential difference, for our purposes, between unheated spores and similar spores that have received a preliminary heat treatment at 85°C for 15 minutes.

TABLE VI

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: Spore Count per ml	Control: % Survivors	Control: Log % Survivors	Irradiated: Spore Count per ml	Irradiated: % Survivors	Irradiated: Log % Survivors
Run 39							
		Heated, but not irradiated			Heated and irradiated (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
		Not heated, not irradiated			Not heated, but irradiated (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.

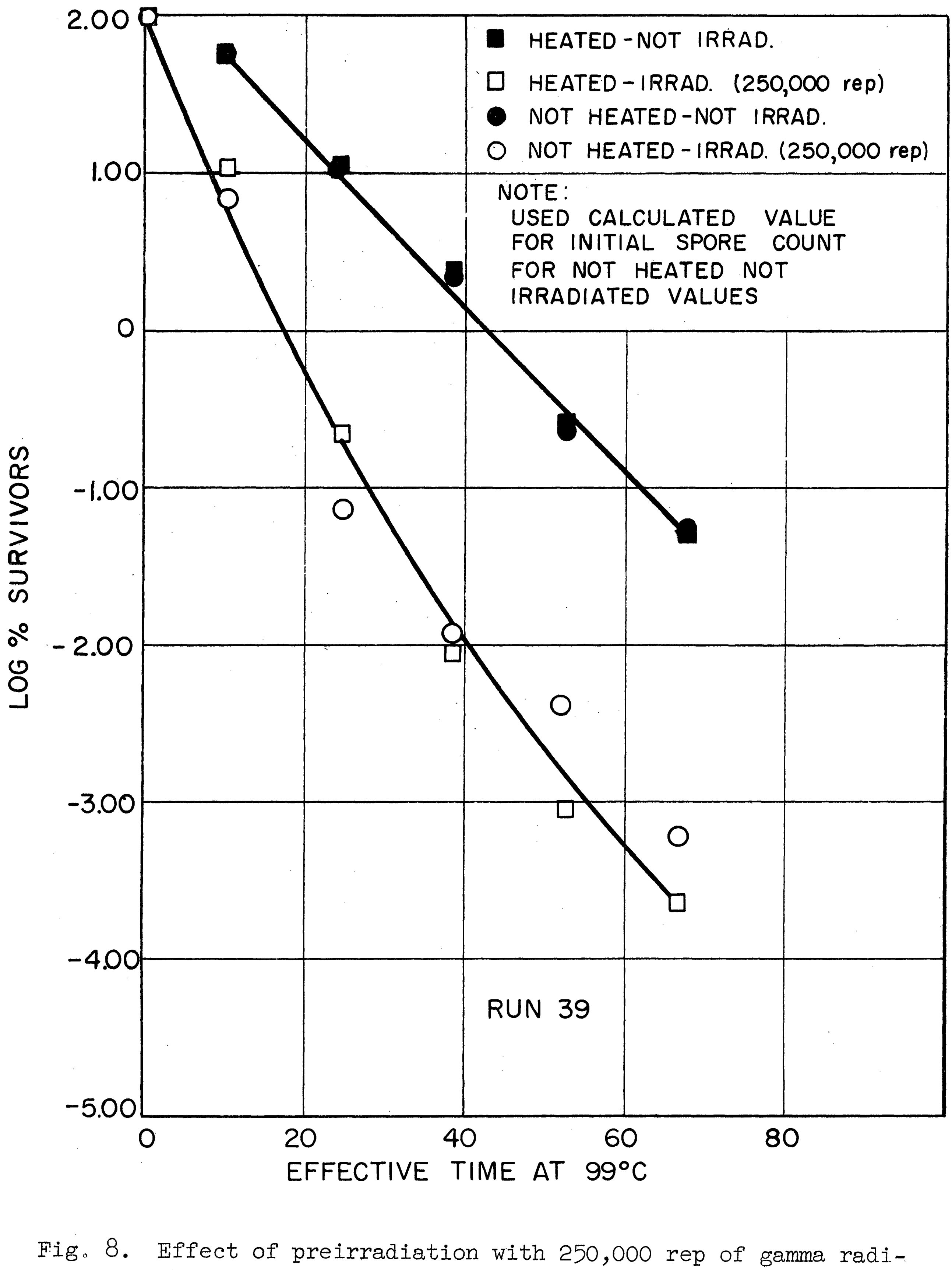

Fig. 8. Effect of preirradiation with 250,000 rep of gamma radiation followed by heating at 99°C on unheated C. botulinum 213B spores and on similar spores that had been heated at 85°C for 15 minutes to kill vegetative cells.

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.[2]

Table VII and Fig. 9 show that when *C. botulinum* 213B spores were suspended in M/15 phosphate buffer at pH 7.0, they were progressively more rapidly killed as the temperature of irradiation was increased from -70 to 95°C. Table VIII and Fig. 10 indicate that the spores of Putrefactive Anaerobe No. 3679 reacted in the opposite manner, since when these spores were suspended in M/15 phosphate buffer at pH 7.0 they were killed more rapidly at irradiation temperatures of 58°C or lower than at temperatures of 80°C and above. This phenomenon is being studied for verification and explanation.

If it should be established that the lethality of gamma radiation for *C. botulinum* spores varies directly with the temperature of irradiation and that PA 3679 spores react oppositely, such information might be of considerable significance in food sterilization. This arises from the fact that *C. botulinum* spores are among the most resistant to gamma radiation; so, from the microbiological standpoint, it might be advantageous to irradiate foods at high temperatures. This would tend to make *C. botulinum* and PA 3679 spores more nearly equal in radiation sensitivity which would cause the spoilage of food to be the first sign of inadequate irradiation rather than toxin production.

Also, the protection afforded *C. botulinum* spores by low temperature should be considered when evaluating suggestions for the prevention of flavor development by irradiation in the frozen state.

TABLE VII

EFFECT OF TEMPERATURE DURING IRRADIATION WITH GAMMA RAYS FROM COBALT-60 ON THE SURVIVAL OF SPORES OF C. BOTULINUM 213B WHEN SUSPENDED IN M/15 PHOSPHATE BUFFER AT pH 7.0

	5°C		30°C		58°C	
Dose,* megarep	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors
0	6,200,000	2.000	2,500,000	2.000	3,500,000	2.000
0.185	2,000,000	1.508	---------	-----	1,300,000	1.570
0.370	5,700,000	0.964	900,000	1.560	5,200,000	1.170
0.550	140,000	0.354	350,000	1.146	100,000	0.456
0.647	---------	-----	110,000	0.644	---------	-----
0.740	41,000	2.820	22,000	-0.055	15,000	-0.369
0.832	---------	-----	1,000	-1.398	---------	-----
0.925	3,900	-2.201	150	-2.222	1,500	-1.369
1.017	150	-3.617	85	-3.469	---------	-----
1.110	3	-4.315	---------	-----	---------	-----

	80°C		95°C		Heat Control		
Dose,* megarep	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors	Hr.	80°C	85°C
0	5,500,000	2.000	2,700,000	2.000	0	5,500,000	2,700,000
0.185	3,200,000	1.765	---------	-----	2	---------	3,000,000
0.370	1,400,000	1.407	2,300,000	1.930	3	---------	2,300,000
0.550	160,000	0.465	14,000	-0.215	4	---------	1,500,000
0.740	7,800	-0.848	45	-1.777	5	3,000,000	1,400,000
0.832	3,500	-1.197	0	-----	-	---------	---------
0.877	1,400	-1.595	0	-----	-	---------	---------

	-70°C		-7°C		27°C	
Dose,** megarep	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors
0	950,000	2.000	520,000	2.000	670,000	2.000
0.227	720,000	1.903	520,000	2.000	530,000	1.898
0.454	350,000	1.522	79,000	1.225	260,000	1.589
0.680	75,000	0.940	9,700	0.314	78,000	1.065
0.794	25,000	0.440	-------	-----	-------	-----
0.907	12,000	-0.060	1,800	-0.523	71,000	1.025
1.020	5,000	-0.246	200	-1.366	-------	-----
1.134	1,500	-0.773	-------	-----	790	-0.928

Frozen control 9.0 x 10^5.

*Dosage rate = 0.185 megarep per hour.

**Dosage rate = 0.227 megarep per hour (vials not immersed in liquid).

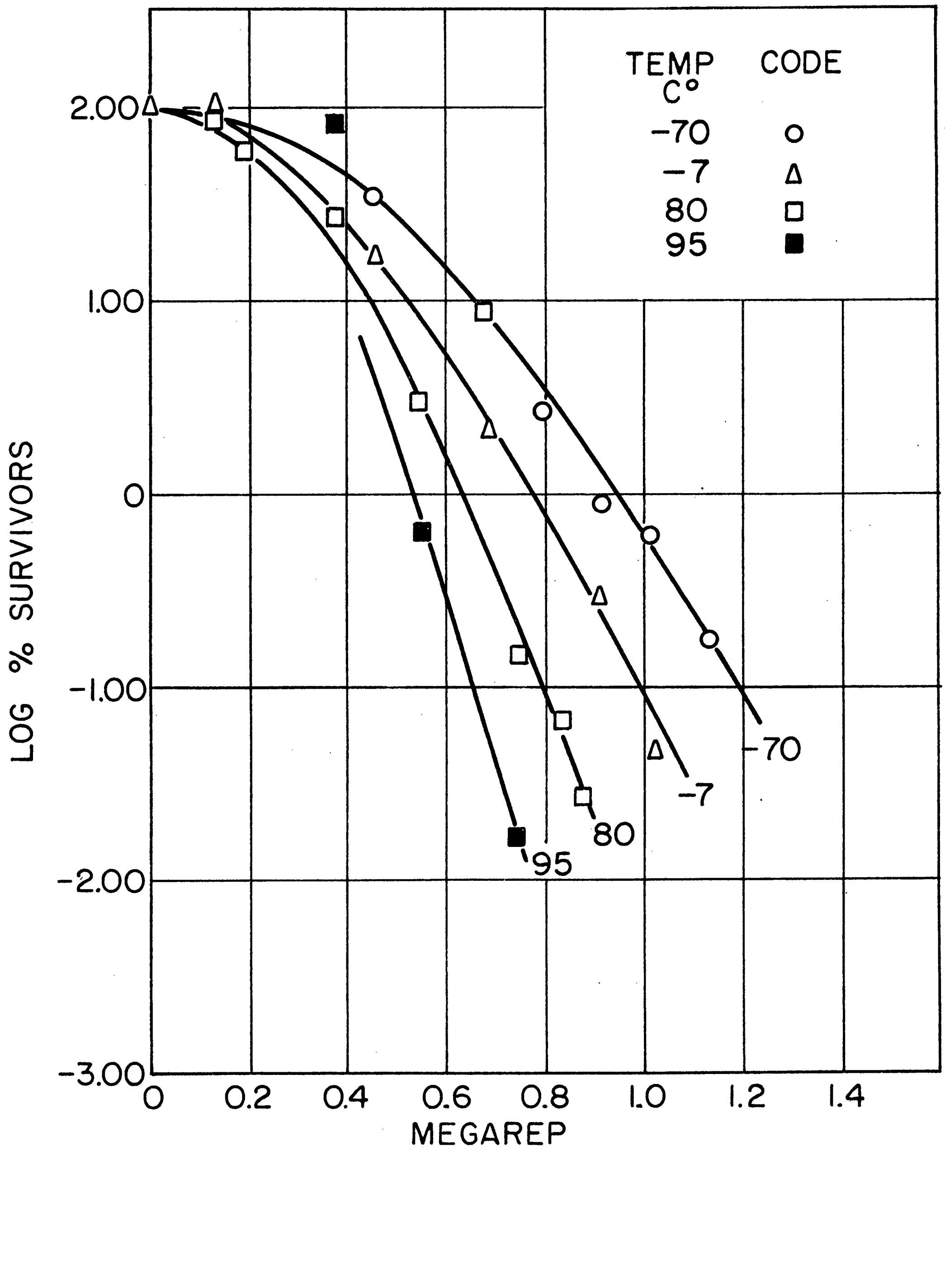

Fig. 9. Effect of temperature during irradiation with gamma rays from cobalt-60 on the survival of spores of C. botulinum 213B suspended in M/15 phosphate buffer at pH 7.0.

TABLE VIII

EFFECT OF TEMPERATURE DURING IRRADIATION WITH GAMMA RAYS FROM COBALT-60 ON THE SURVIVAL OF SPORES OF PA 3679 WHEN SUSPENDED IN M/15 PHOSPHATE BUFFER AT pH 7.0

	5°C		30°C		56°C	
Dose,* megarep	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors
0	850,000	2.000	280,000	2.000	400,000	2.000
0.185	700,000	1.911	-------	-----	300,000	1.875
0.370	220,000	1.409	54,000	1.286	180,000	1.653
0.550	15,000	0.243	13,000	0.668	35,000	0.942
0.647	-------	-----	1,900	-0.157	-------	-----
0.740	900	-0.979	1,400	-0.301	2,700	-0.171
0.832	-------	-----	220	-1.103	-------	-----
0.925	160	-1.731	20	-2.146	-------	-----
1.017	35	-2.392	4	-2.845	-------	-----
1.110	1	-3.935	-------	-----	-------	-----

*Dosage rate = 0.185 megarep per hour

	58°C		80°C		85°C	
Dose,* megarep	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors	Number of Spores	Log % Survivors
0	480,000	2.000	1,100,000	2.000	700,000	2.000
0.185	480,000	2.000	950,000	1.937	-------	-----
0.370	210,000	1.640	530,000	1.684	470,000	1.826
0.550	59,000	1.089	140,000	1.104	410,000	1.767
0.647	-------	-----	---------	-----	140,000	1.301
0.740	1,100	-0.641	40,000	0.558	120,000	1.235
0.832	-------	-----	10,000	-0.041	55,000	0.895
0.925	120	-1.602	4,000	-0.439	13,400	0.267
1.017	-------	-----	---------	-----	-------	-----
1.110	-------	-----	---------	-----	-------	-----

	95°C		Heat Control PA 3679		
Dose, megarep	Number of Spores	Log % Survivors	Hr	85°C	95°C
0	1,200,000	2.000	-	-------	---------
0.185	---------	-----	0	700,000	1,200,000
0.370	900,000	1.875	2	950,000	1,400,000
0.550	570,000	1.676	3	-------	1,600,000
0.647	---------	-----	4	810,000	1,400,000
0.740	140,000	1.068	5	-------	1,200,000
0.832	51,000	0.628	5.5	790,000	---------
0.925	30,000	0.398	-	-------	---------
1.017	10,000	-0.061	-	-------	---------
1.110	3,100	-0.558	-	-------	---------

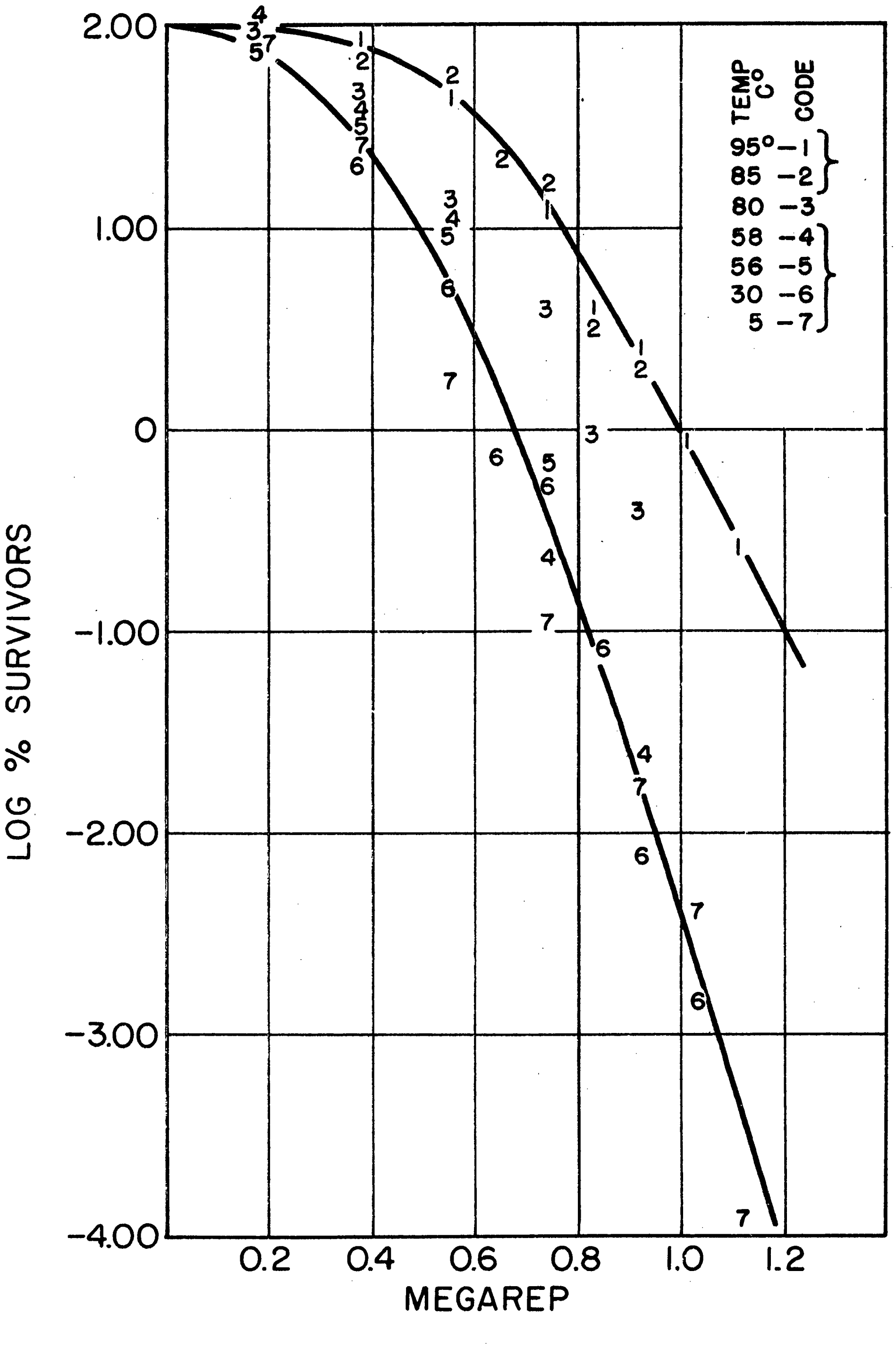

Fig. 10. Effect of temperature during irradiation with gamma rays from cobalt-60 on the survival of spores of PA 3679 suspended in M/15 phosphate buffer at pH 7.0.

B. THE EFFECT OF TEMPERATURE DURING IRRADIATION ON THE SENSITIZATION 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 IX and Figs. 11 and 12 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 PA 3679 spores show little if any decrease in numbers when heated for 1 hour at 99°C. 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.

2. *C. botulinum* 62A.—*C. botulinum* 62A spores were suspended in M/15 phosphate buffer at pH 7.0, distributed into vials, and then irradiated with 500,000 rep of gamma radiation from cobalt-60 while held at 5 or -70°C. Thermal-death time tests were then carried out on the irradiated spores by holding them at 99°C for the periods of time indicated.

The data shown in Table X and Fig. 13 show that *C. botulinum* spores are killed at essentially the same rate by subsequent heat treatment at 99°C in both instances. This tentatively indicates that irradiation at -70°C does not alter the phenomenon of sensitization to heat developed by preirradiation. It would thus appear that food could be irradiated in the frozen condition and still be processed by subsequent heat treatment in such a way as to take advantage of the decreased heat resistance of bacterial spores following irradiation.

TABLE IX

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
A) Irradiated at 5°C and heated for 1 hour at 99°C			
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
B) Irradiated at 95°C and heated 1 hour at 99°C			
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
1,100,000	36	0.00328	-2.4840
1,100,000	8	0.000726	-3.1391

Note: See Table VIII for control data of radiation alone.

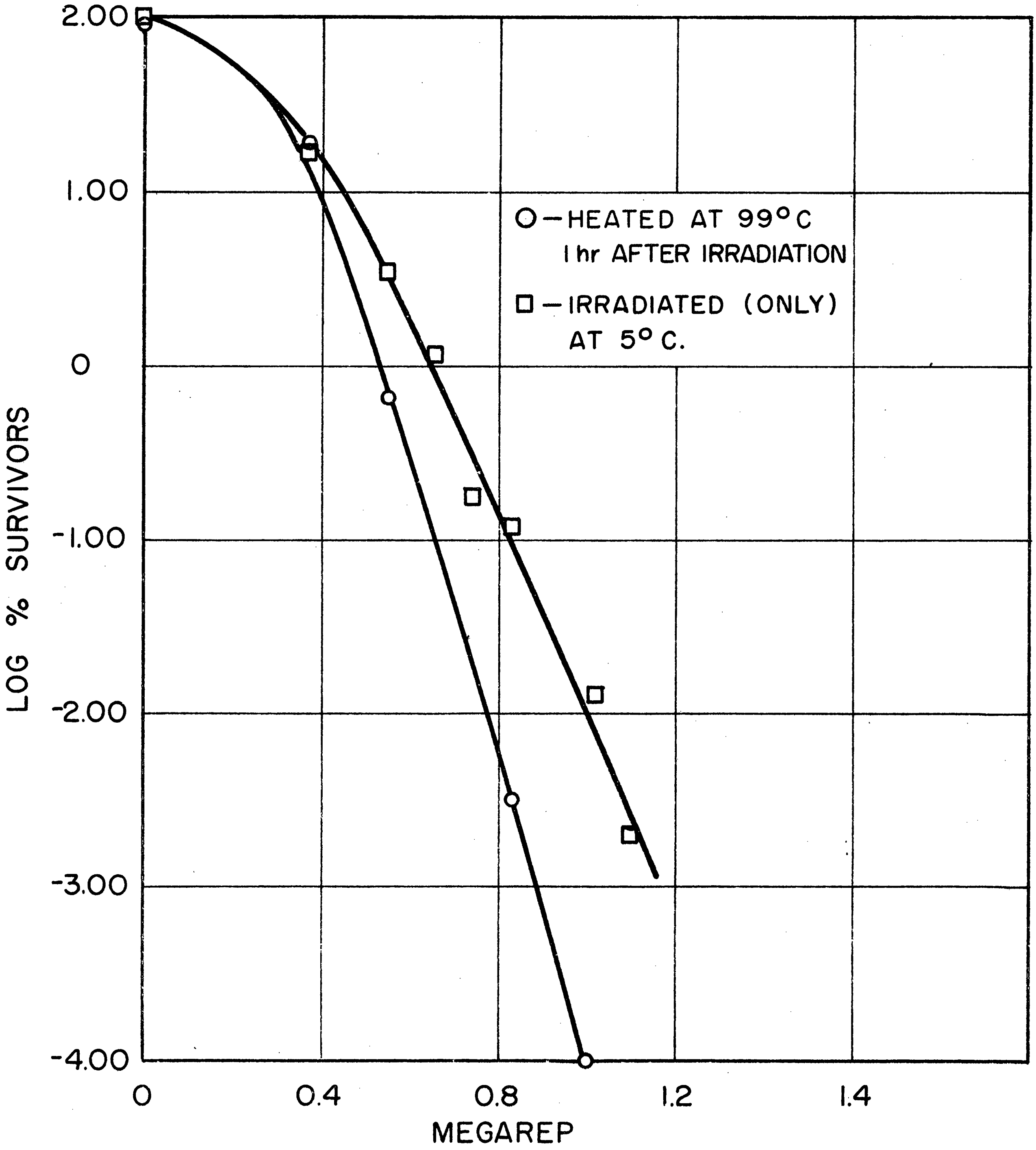

Fig. 11. Effect of irradiation at 5°C followed by heating for 1 hour at 99°C on the survival of PA 3679 spores in phosphate buffer at pH 7.0.

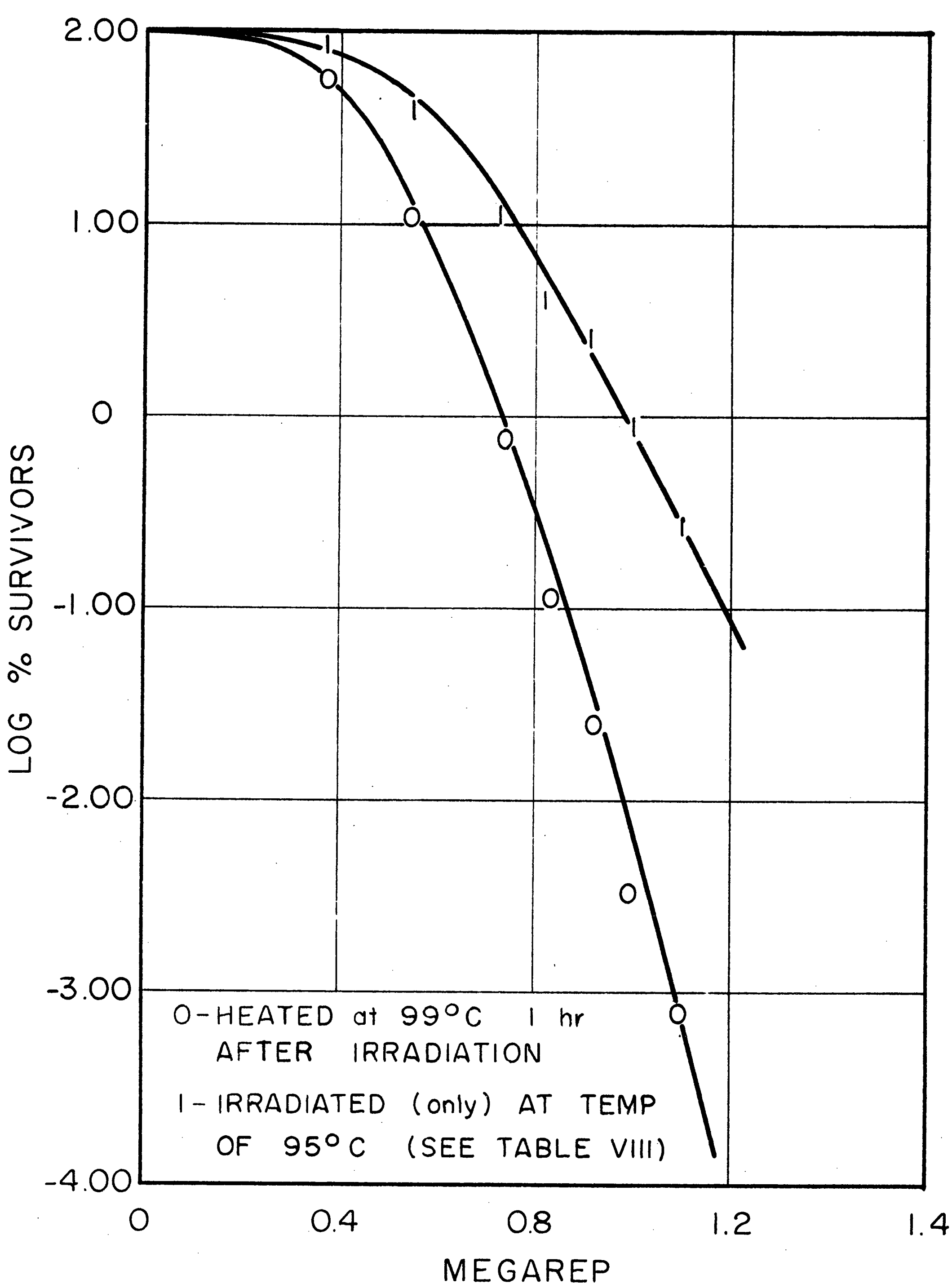

Fig. 12. Effect of irradiation at 95°C followed by heating for 1 hour at 99°C on the survival of PA 3679 spores in phosphate buffer at pH 7.0.

TABLE X

EFFECT OF TEMPERATURE DURING IRRADIATION ON THE SUBSEQUENT RESISTANCE OF C. BOTULINUM 62A SPORES TO HEATING AT 100°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
A) (500,000 rep at -70°C)							
0	0	370,000	100.0	2.00	90,000	100.0	2.00
5	2.6				58,000	64.5	1.4416
10	7.6	220,000	59.5	1.774	16,000	17.7	1.150
15	12.6	150,000	40.5	1.608	7,700	8.55	0.7161
20	17.6	110,000	29.8	1.474	290	0.322	-0.4939
30	27.6	26,000	7.03	0.847			
40	37.6	9,900	2.68	0.428			
50	47.6	3,000	0.811	-0.091			
60	57.6	1,100	0.298	-0.526			
B) (500,000 rep at +5°C)							
0					86,000	100.0	2.00
5					71,000	82.5	1.9165
10		Same control as (A)			4,300	5.0	0.699
15					1,000	1.16	0.065
20					35	0.04	-1.398

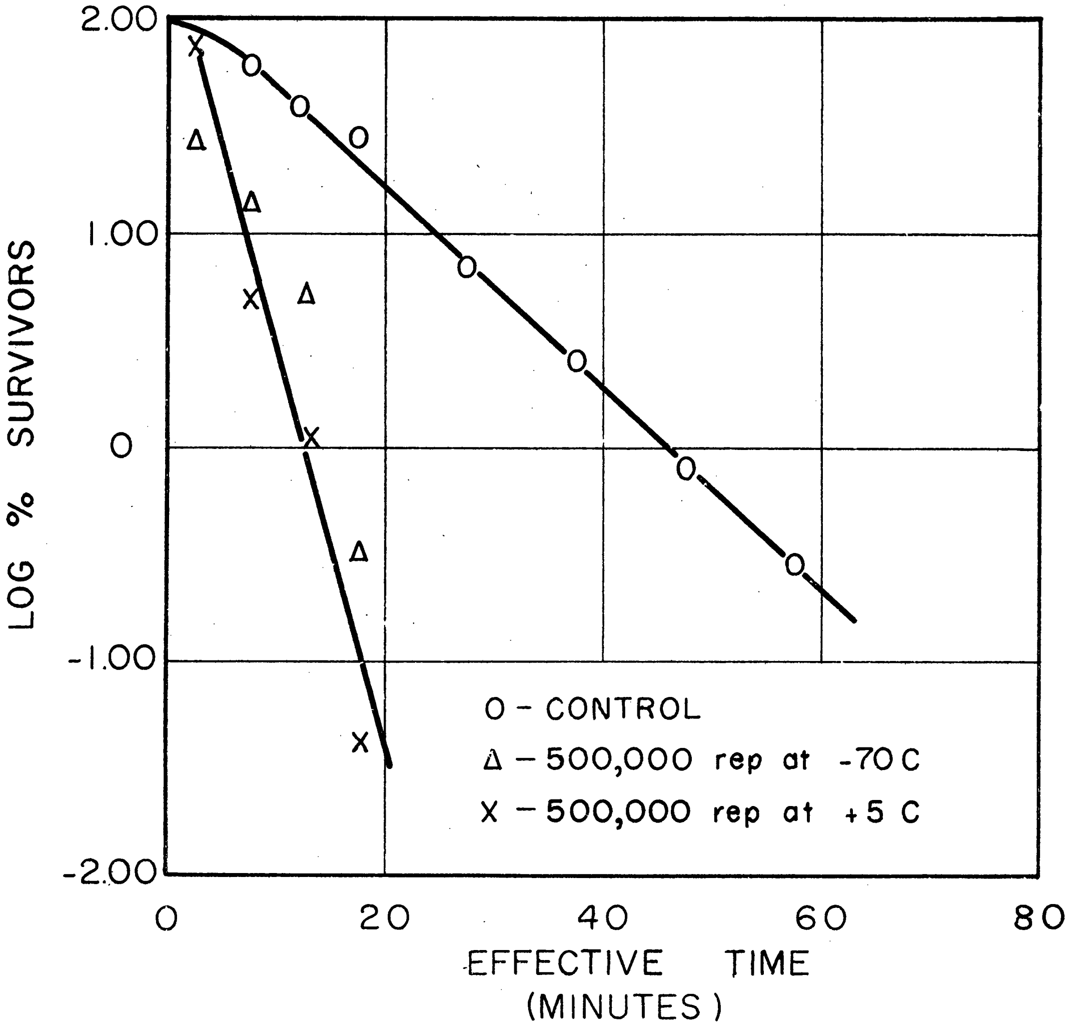

Fig. 13. Effect of temperature during irradiation on the subsequent resistance of C. botulinum 62A spores to heating at 100°C while suspended in M/15 phosphate buffer at pH 7.0.

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

Morgan and Reed[1] have reported that certain media components affect the rate at which bacterial spores are killed by gamma radiation. Also, work in this laboratory on the present QMC contract has shown that gelatin and nutrient broth reduce the lethality of gamma radiation for the spores of C. botulinum. It is evident that information regarding the effect of such media components on the lethality of gamma radiation for bacterial spores is needed to interpret the effectiveness of ionizing radiations for food sterilization. With this in mind, several chemicals have been investigated in a preliminary fashion to determine their effect on the lethality of gamma radiation for C. botulinum 62A spores.

A. MATERIALS AND METHODS

A 1:5 dilution of a 10^8-per-ml spore suspension was prepared in M/15, pH 7.02, phosphate buffer. One ml of the suspension was added to each 20 ml of the solution to be used for irradiation.

Organism: C. botulinum 62A

Control: Spore suspension in M/15, pH 7.02, phosphate buffer

Chemicals:

0.5% lecithin
0.5% ferrous sulfate
0.5% lead (plumbous) chloride
0.5% nicotinic acid
0.5% riboflavin
0.5% biotin
0.5% glutathione
0.5% vitamin A*
0.5% vitamin K*

*For the fat-soluble vitamins, the phosphate buffer spore suspensions were mixed with the oil solution of the vitamin and allowed to stand from 4:00 p.m. on one day until 9:00 a.m. the following morning. The suspensions were placed in a dark cabinet at room temperature. The oil and aqueous emulsion was then separated by centrifugation and the spores (in the aqueous portion) were pipetted off into phosphate buffer for irradiation purposes.

0.5% gelatin
0.5% l-lysine monohydrochloride
0.5% dl-methionine

All except lead chloride were prepared in M/15, pH 7.02, phosphate buffer (lead chloride in sterile water).

B. IRRADIATION PROCEDURE

Each of the chemicals was weighed out aseptically into sterile weighing bottles and then added to 20 ml of sterile M/15 phosphate buffer at pH 7.0 to provide a 0.5% concentration. Then 1 ml of 1:5 dilution of C. botulinum 62A spores was added to each flask. Samples of 4 ml of the suspension were placed in sterile glass vials and heat-sealed. They were then irradiated by the cobalt-60 gamma radiation source following which the number of surviving spores were counted in pork infussion agar in Prickett tubes.

C. DISCUSSION

The data shown in Table XI and Figs. 14a through 14e indicate that many chemicals reduce the lethality of gamma radiation for spores of C. botulinum 62A. Among the more important of these are reducing substances and chemicals containing sulfur.

This work will be continued next year in an attempt to establish the mechanisms of spore protection involved.

TABLE XI

EFFECT OF VARIOUS CHEMICALS ON THE LETHALITY
OF GAMMA RADIATION FROM COBALT-60 ON THE SPORES
OF C. BOTULINUM 62A SUSPENDED IN M/15 PHOSPHATE BUFFER AT pH 7.0

Control (M/15 Phosphate Buffer at pH 7.0)				Chemical in M/15 Phosphate Buffer at pH 7.0		
Dosage, megarep	Spores per ml	% Survivors	Log % Survivors	Spores per ml	% Survivors	Log % Survivors
				Vitamin A		
0	520,000	100	2.00	680,000	100	2.00
0.342	143,000	27.50	1.439	198,000	29.12	1.464
0.672	7,200	1.38	0.140	14,300	2.10	0.322
				Vitamin K		
0.0				480,000	100	2.00
0.342	"	"	"	200,000	41.67	1.620
0.672				8,400	1.75	0.243
				Lecithin		
0.0				620,000	100.0	2.00
0.342	"	"	"	240,800	40.0	1.602
0.672				20,000	3.23	0.509
				Ferrous Sulfate		
0				580,000	100.0	2.000
0.342	"	"	"	208,000	35.86	1.555
0.672				60,000	10.34	1.015
				Plumbous Chloride		
0				650,000	100.0	2.00
0.342	"	"	"	165,000	25.38	1.405
0.672				3,000	0.46	-0.335
				Biotin		
0				450,000	100.0	2.00
0.342	"	"	"	175,000	38.89	1.59
0.672				14,400	3.20	0.505
				Riboflavin		
0				650,000	100.0	2.00
0.342	"	"	"	250,000	38.31	1.583
0.672				26,000	4.00	0.602

TABLE XI (concluded)

	Control (M/15 Phosphate Buffer at pH 7.0)			Chemical in M/15 Phosphate Buffer at pH 7.0		
Dosage, megarep	Spores per ml	% Survivors	Log % Survivors	Spores per ml	% Survivors	Log % Survivors
				Nicotinic Acid		
0	520,000	100	2.00	420,000	100.0	2.00
0.342	143,000	27.50	1.439	203,000	48.33	1.684
0.672	7,200	1.38	0.140	17,100	4.07	0.610
				Glutathione		
0	1,030,000	100.0	2.00	980,000	100.0	2.00
0.169	765,000	74.27	1.871	965,000	98.47	1.993
0.338	500,000	48.54	1.686	775,000	79.08	1.898
0.676	53,000	5.146	0.711	264,500	27.00	1.431
0.845	8,050	0.782	-0.107	104,000	10.61	1.026
1.014	1,075	0.104	-0.983	24,500	2.50	0.398
				Gelatin		
0	610,000	100	2.00	970,000	100.0	2.00
0.169	453,100	74.28	1.871	745,000	76.8	1.885
0.338	315,000	51.64	1.713	510,000	52.58	1.721
0.676	56,000	9.18	0.963	156,000	16.08	1.206
0.845	8,050	1.32	0.121	64,000	6.598	0.819
1.014	1,075	0.176	-0.754	25,000	2.577	0.411
				l-Lysine		
0				530,000	100.0	2.00
0.169				--	--	--
0.338	"	"	"	400,000	75.47	1.878
0.676				84,000	15.85	1.200
0.845				18,250	3.44	0.537
1.014				3,950	0.745	-0.128
				dl-Methionine		
0				540,000	100.0	
0.169				--	--	2.00
0.338	"	"	"	365,000	67.60	1.830
0.676				179,500	33.24	1.522
0.845				93,000	17.22	1.236
1.014				35,000	6.48	0.812

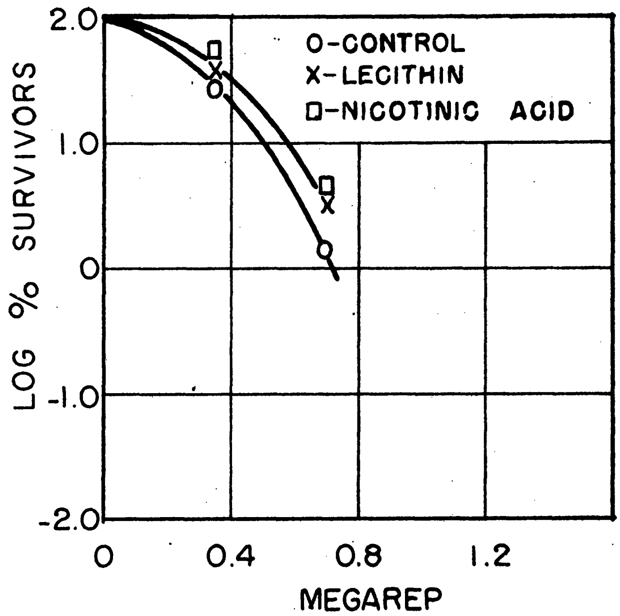

Fig. 14a

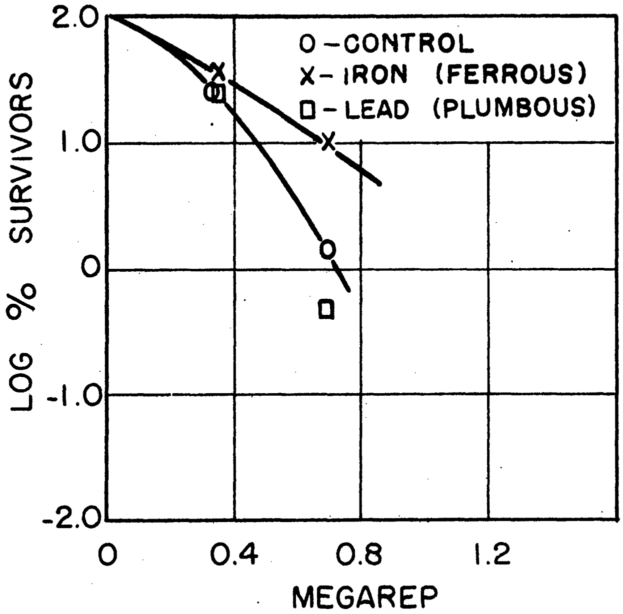

Fig. 14b

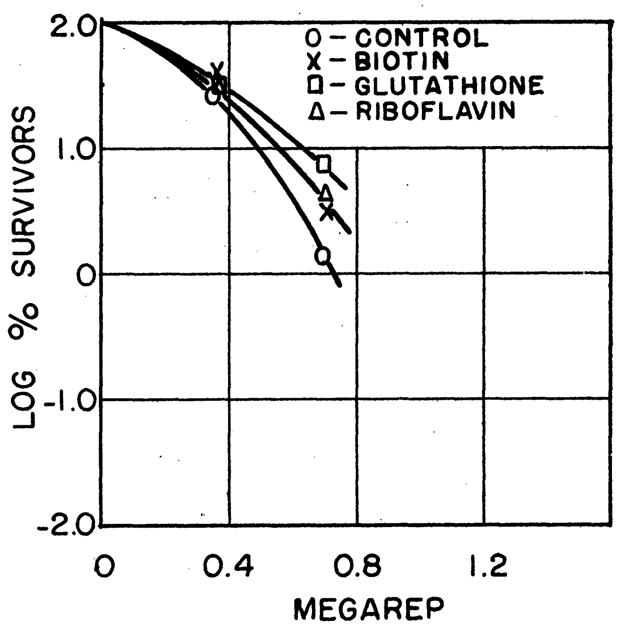

Fig. 14c

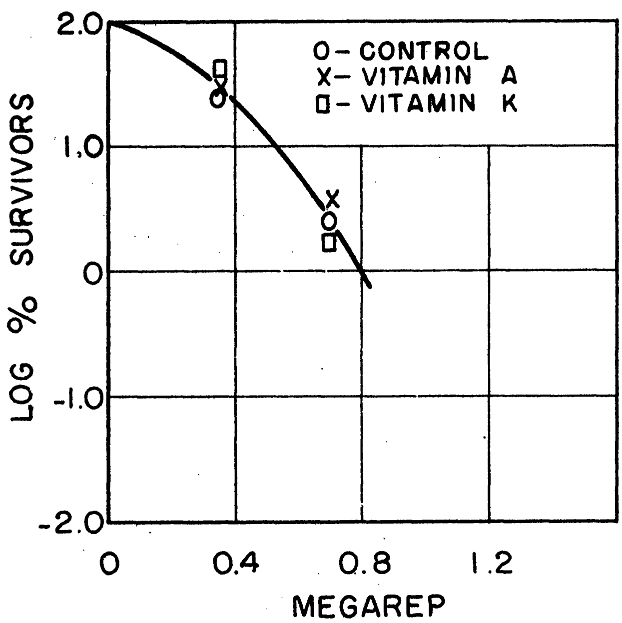

Fig. 14d

Effect of various chemicals incorporated into M/15 phosphate buffer at pH 7.0 on the lethality of gamma radiation from cobalt-60 on the spores of C. botulinum 62A suspended in the solutions so obtained.

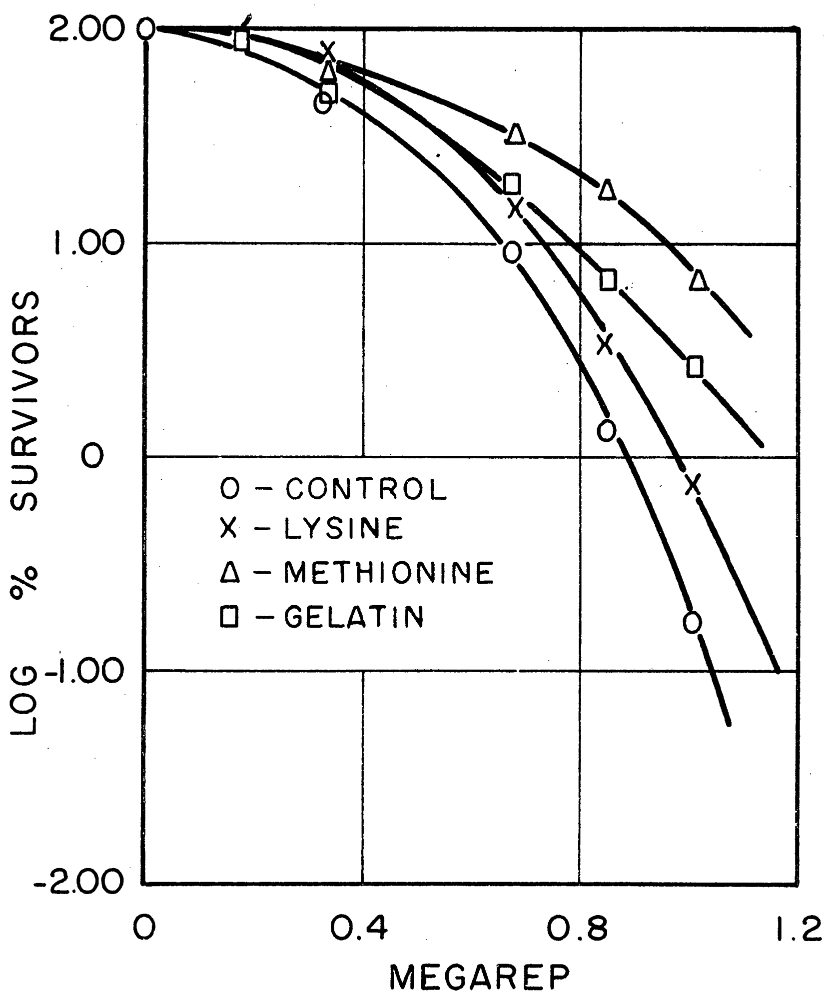

Fig. 14e

Effect of various chemicals incorporated into M/15 phosphate buffer at pH 7.0 on the lethality of gamma radiation from cobalt-60 on the spores of C. botulinum 62A suspended in the solutions so obtained.

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