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COMBINED USE OF HEAT AND RADIATION TREATMENT FOR STERILIZATION OF FOODS

Period: February 1, 1957, to March 31, 1957

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SUMMARY

Combined irradiation and heat processing treatments required to sterilize cooked ground beef packed in No. 1 picnic cans and then inoculated with 300 PA-3679 spores per can were studied. Without irradiation, an F_0 of approximately six was required to sterilize the meat; with irradiation alone, between 1.750 and 2.020 megarep of gamma radiation were required; but when irradiation and heat were combined, cans of meat receiving more than one megarep preirradiation were subsequently sterilized with F_0 values less than two.

It is pointed out that PA-3679 spores are much more resistant to combined irradiation and heat processing than are C. botulinum spores. This is important from a public health viewpoint because there is far more danger from botulism when only irradiation is used.

Catalase present in the suspending medium during irradiation significantly reduced the lethal action of gamma radiation from cobalt-60 for anaerobic bacterial spores. This finding supports the theory that the lethal action of ionizing radiations is at least partially due to secondary effects of the irradiation. Furthermore, such protection of anaerobic bacterial spores is important when sterilization of foods containing catalase is considered since it will likely increase the dosage required.

EFFECT OF PREIRRADIATION OF CANNED GROUND BEEF INOCULATED WITH PA-3679 SPORES ON THE F_0 SUBSEQUENTLY REQUIRED FOR STERILIZATION

The combined irraidation-heat processing treatments required to sterilize cooked ground beef in No. 1 picnic tin cans inoculated with 300 PA-3679 spores per can were studied. The techniques in use were previously outlined in the 1956 Annual Report and in Progress Report No. 9 of this project.

Table I includes data from runs PA-26 through PA-33, all of which were carried out under similar conditions to those indicated. Results of these runs are summarized in Table II and in the figure for inoculated packs treated as follows:

Can size—No. 1 picnic (211 x 400)
Product—Precooked ground beef
Inoculum—300 PA-3679 spores per can
Preirradiation—As indicated
Processing temperature—230°F
Incubation temperature—84°F

Without irradiation, an F_0 of approximately six was required to sterilize the precooked ground beef; with irradiation alone, between 1.750 and 2.020 megarep were needed; but when irradiation and heat were combined, cans of meat receiving more than one megarep preirradiation were subsequently sterilized with F_0 values of less than two.

It was previously pointed out* that the high heat-resistance of PA-3679 spores restores the desirable processing resistance between C. botulinum and PA-3679 spores, i.e., in canned meat, the latter are more resistant to combined irradiation-heat processing than the former. This safety factor against botulism exists in present commercial heat-processing methods and also in the combined irradiation-heat processing treatments reported here.

Parallel with the data previously reported** for C. botulinum 213B spore packs, there is an appreciable effect of numbers of PA-3679 spores on the F_0 value required to sterilize canned ground beef after any selected limit of preirradiation up to about one megarep. For example, it was previously reported* that with 10,000 PA-3679 spores per can of cooked ground beef, an F_0 of four was required for sterilization following one megarep of irradiation with gamma rays; after a similar amount of irradiation, cans of

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* Progress Report No. 9

** Annual Report for 1956

ground beef containing only 300 PA-3679 spores required an F_0 of two for this purpose.

It is also fortunate and important that the combined heat and irradiation sterilization treatments required for 300 PA-3679 spores per can of cooked ground beef are much higher than those reported for even 5,000,000 C. botulinum** 213B spores per can of similar food. Therefore, a combined irradiation-heat process designed to prevent spoilage of the meat by PA-3679 spores will present the same type of safety factor towards possible botulism poisoning inherent in the usual heat processing technique now in common industrial use for canned meat.

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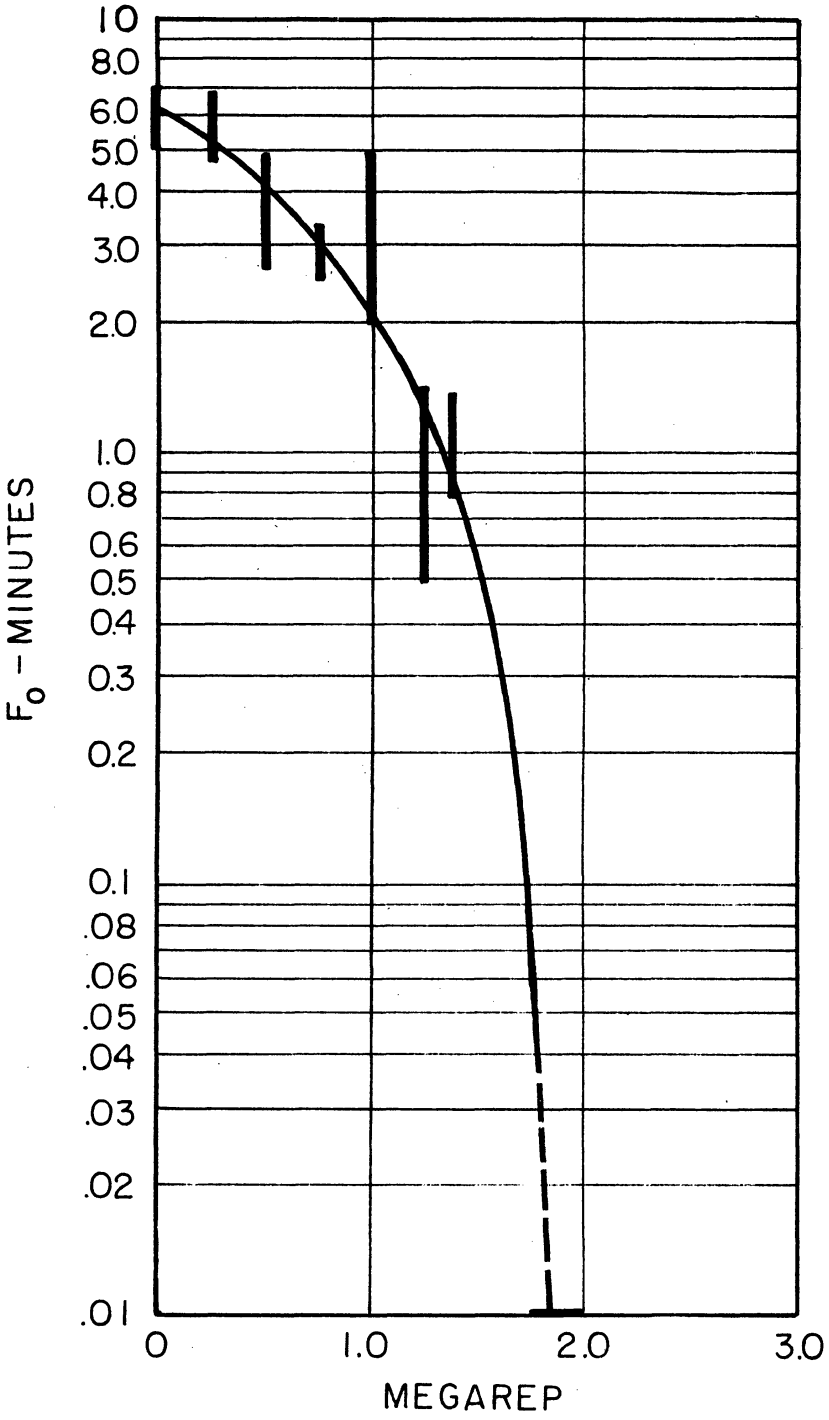


F_o required to sterilize cooked ground beef packed in No. 1 picnic cans, inoculated with 300 PA-3679 spores per can, and irradiated with gamma rays from cobalt-60 before heat-processing at 230°F.

TABLE I - F_o Value Required to Sterilize Ground Beef in No. 1 Picnic Tin Cans, Previously Inoculated with Approximately 10,000 PA-3679 Spores per Can and Then Processed at 230°F.

Run No. PA-26—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 0.500 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 1.97	1	5
Can 2, 1.97	2	5
Can 3, 1.97	3	4
	4	5
Can 1, 2.87	5	6
Can 2, 2.83	6	9
Can 3, 2.49	7	4
	8	45
Can 1, 7.42	9	-
Can 2, 7.43	10	-
Can 3, 6.51	11	-
	12	
Can 1, 4.50	13	-
Can 2, 4.81	14	-
Can 3, 4.85	15	-
	16	-

Conclusion: Sterilization was accomplished with 0.500 megarep irradiation followed by an F_o between 2.5 and 4.9.

TABLE I (Continued)

Run No. PA-27—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - none
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Controls — See Run No. PA-26		
Can 1, 0.63	1	4
Can 2, 0.55	2	4
Can 3, 0.62	3	4
	4	4
Can 1, 0.36	5	3
Can 2, 0.28	6	3
Can 3, 0.29	7	3
	8	3
Can 1, 8.10	9	-
Can 2, 8.46	10	-
Can 3, 7.77	11	-
	12	-
Can 1, 1.91	13	5
Can 2, 1.76	14	5
	15	4
	16	4
Can 1, 1.02	17	4
Can 2, 0.59	18	3
	19	4
	20	4
Can 1, 5.01	21	-
Can 2, 5.01	22	-
	23	17
	24	-
Can 1, 3.14	25	7
Can 2, 3.14	26	5
Can 3, 3.14	27	6
	28	7
Can 1, 4.07	29	8
Can 2, 4.31	30	6
Can 3, 3.53	31	6
	32	5
Can 1, 7.05	33	-
Can 2, 7.05	34	-
Can 3, 7.05	35	-
	36	-

Conclusion: Sterilization was accomplished with an F_o between 5.0 and 7.1.

TABLE I (Continued)

Run No. PA-28—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 1.000 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 0.90	1	5
Can 2, 0.90	2	-
Can 3, 0.46	3	10
	4	8
Can 1, 1.8	5	-
Can 2, 1.1	6	7
	7	-
	8	-
Can 1, 3.50	9	-
Can 2, 4.68	10	-
Can 3, 4.95	11	-
	12	-
Can 1, 2.0	13	-
Can 2, 2.6	14	-
Can 3, 2.9	15	-
	16	10

Conclusion: Sterilization was accomplished with 1.000 megarep of gamma radiation followed by an F_o between 2.0 and 5.0.

TABLE I (Continued)

Run No. PA-29—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 0.750 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Controls: — See Run No. PA-28		
Can 1, 1.84	1	6
Can 2, 1.43	2	6
Can 3, 1.65	3	5
	4	5
Can 1, 2.56	5	-
Can 2, 2.56	6	10
Can 3, 2.56	7	11
	8	-
Can 1, 0.74	9	5
Can 2, 1.02	10	5
Can 3, 0.53	11	5
	12	5
Can 1, 3.48	13	-
Can 2, 3.48	14	-
Can 3, 3.68	15	-
	16	-

Conclusion: Sterilization was accomplished by 0.750 megarep of gamma radiation followed by an F_o between 2.6 and 3.7.

TABLE I (Continued)

Run No. PA-30—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 1.250 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 2.32	9	-
Can 2, 1.47	10	-
Can 3, 1.70	11	-
	12	-
Can 1, 0.38	13	5
Can 2, 0.24	14	4
Can 3, 0.12	15	4
	16	4
Can 1, 0.98	17	-
Can 2, 0.98	18	5
Can 3, 0.48	19	-
	20	-
Can 1, 1.29	21	-
Can 2, 1.29	22	-
Can 3, 1.50	23	-
	24	-

Conclusion: Sterilization was accomplished by 1.250 megarep of gamma radiation followed with an F_o between 0.48 and 1.50.

TABLE I (Continued)

Run No. PA-31—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Incubation Temperature - 85°F

Radiation Dosage (megarep)	Can No.	Days to Gas Formation
Controls — See Run No. PA-30		
1.750	1	-
	2	5
	3	-
	4	5
2.160	17	-
	18	-
	19	-
	20	-
1.250	9	3
	15	3
	12	4
	13	3
1.390	10	5
	11	4
	14	5
	16	4
2.020	5	-
	6	-
	7	-
	8	-

Conclusion: Sterilization was accomplished with between 1.750 and 2.020 megarep of gamma radiation.

TABLE I (Continued)

Run No. PA-32—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 0.250 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Inoculated Controls	1	3
	2	3
	3	3
	4	3
Can 1, 3.28	1	6
Can 2, 3.49	2	5
	3	6
	4	5
Can 1, 4.83	5	7
Can 2, 4.83	6	7
Can 3, 4.52	7	11
	8	9
Can 1, 6.93	9	-
Can 2, 6.61	10	-
Can 3, 6.66	11	-
	12	-
Can 1, 7.65	13	-
Can 2, 8.09	14	-
Can 3, 8.09	15	-
	16	-

Conclusion: Sterilization was accomplished be 0.250 megarep of gamma radiation followed by an F_o between 4.5 and 6.9.

TABLE I (Concluded)

Run No. PA-33—Can Size - No. 1 Picnic (211 x 400)
Product - Cooked Ground Beef
Inoculum - 300 PA-3679 spores per can
Preirradiation - 1.350 megarep
Processing Temperature - 230°F
Incubation Temperature - 85°F

F_o	Can No.	Days to Gas Formation
Noninoculated Controls	1	-
	2	-
	3	-
	4	-
Can 1, 0.31	1	5
Can 2, 0.31	2	-
	3	-
	4	7
Can 1, 1.46	5	-
Can 2, 1.46	6	-
	7	-
	8	-
Can 1, 0.77	9	7
Can 2, 1.13	10	-
Can 3, 1.13	11	-
	12	-
Can 1, 0.58	13	-
Can 2, 0.42	14	-
Can 3, 0.54	15	-
	16	5

Conclusion: Sterilization was accomplished by 1.350 megarep of gamma radiation followed with an F_o between 0.77 and 1.46.

THE EFFECT OF CATALASE ON THE LETHALITY OF Co-60 GAMMA RADIATION FOR CERTAIN ANAEROBIC BACTERIAL SPORES

Two explanations are offered for the lethal action of ionizing radiations on living cells. The first postulates a direct action of the radiations on genetic material; the other assumes that an initial change takes place in the medium or at some non-genetic locus and that this change brings about lethal effects in the cell. The work of Hollander et al. (1951) and Burnett et al. (1951) support the latter theory. Similarly, the work presented in this paper further supports the indirect action theory by describing a protective effect of catalase for anaerobic bacterial spores when catalase is present in the suspending medium during irradiation. Obviously, such protection of anaerobic bacterial spores will probably increase the amount of ionizing radiations required to sterilize foods containing catalase.

MATERIALS AND METHODS

Spores used in this work were grown, harvested, and suspended in distilled water as described by Kempe et al. (1954).

Immediately prior to use in an experiment, the stock spore suspensions were shaken with glass beads for five minutes to disperse the spore clumps. The desired quantity of spores was next pipetted into a sterile test tube and heated at 85°C for 15 minutes to kill the vegetative cells. The spore suspension was then diluted into the final solutions to be irradiated.

The control for these experiments was sterile phosphate buffer to which only the spores of either Clostridium botulinum 213-B or Putrefactive anaerobe No. 3679 were added.

Purified crystalline catalase for this work was obtained from General Biochemicals, Inc., Chagrin Falls, Ohio. For use in an experiment, phosphate buffer (pH 7.02) was sterilized by autoclaving. Following this, 60 mgm of catalase was added to 14.5 ml of the cooled experimental solution and then 0.5 ml of a spore suspension was added to both the control and the catalase solutions.

Four ml quantities of these preparations were next aseptically pipetted into sterile 5 ml glass vials which were finally sealed in an oxygen flame. Irradiation was carried out in an ice water bath in the

center well of the large cobalt-60 source in the Fission Products Laboratory at The University of Michigan.

After completion of the irradiation, a sample from the irradiated or control (0 hours radiation) vial was withdrawn, diluted to the proper spore concentration, and counted using techniques previously described by Reed et al. (1951).

RESULTS AND DISCUSSION

The survival of spores of Clostridium botulinum 213-B after varying exposures to Co-60 gamma radiation either in a phosphate buffer solution or in a phosphate buffer solution containing catalase is shown in the table and in the figure. In the control solution, only 35 spores per ml remained viable after 8 hours radiation from an original population of 15,000,000 per ml. On the other hand, in the solution containing catalase, 59,000 spores per ml were viable after 8 hours irradiation from an original population of 16,900,000 per ml. This represents a 1500 fold increase in survival caused by the addition of catalase.

Similarly, the effect of catalase on the lethality of Co-60 gamma radiation for the spores of Putrefactive anaerobe No. 3679 is shown in the table and the figure. Here only 145 spores per ml were viable after 7 hours irradiation in the control solution as compared to 179,500 spores per ml after a similar dosage of gamma radiation in the solution containing catalase. This represents a 1250 fold increase in the survival ratio.

Since many investigators, including Allen (1954), have reported that hydrogen peroxide is developed during irradiation of water by gamma rays, and since Curran and Evans (1940) reported that the sporicidal action of hydrogen peroxide could be dissipated by catalase, the inference is drawn that catalase protected the anaerobic spores tested in this work by destroying hydrogen peroxide that was produced in situ by the irradiation treatment. This is evidence for the indirect lethal action of radiation on anaerobic bacterial spores. The magnitude of the observed effect suggests that the sporicidal action of gamma radiation may be largely indirect in nature.

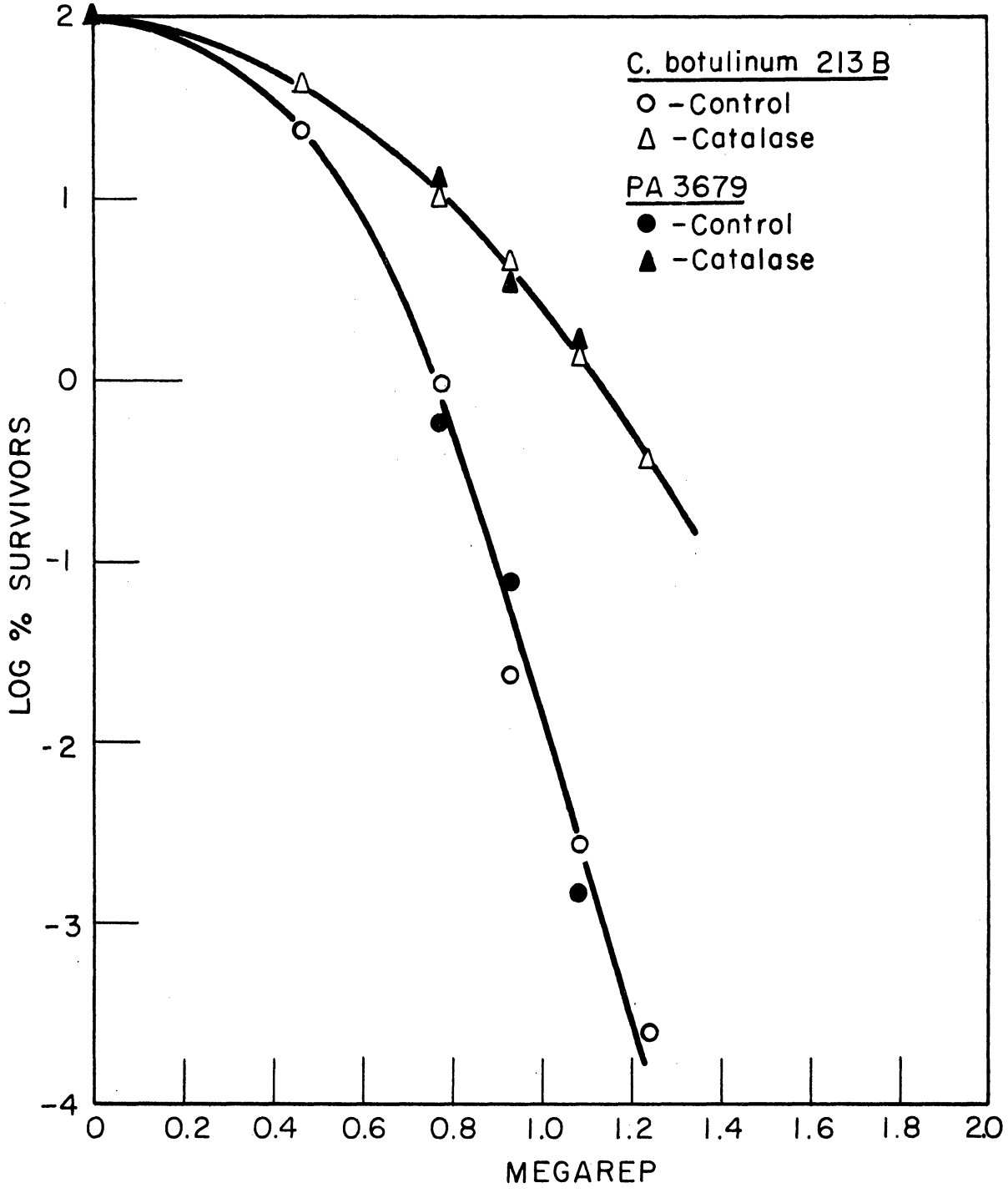
In any event, the protection of anaerobic bacterial spores against the lethal action of ionizing radiations by catalase must be considered when the sterilization of raw foods by such radiations is contemplated.

TABLE II

THE EFFECT OF CATALASE ON THE LETHALITY OF GAMMA RADIATION FROM COBALT-60 FOR ANAEROBIC BACTERIAL SPORES

	No. of Hours Radiation	Rep*	No. of Organisms Surviving per ml	Log % Survivors
a) C. botulinum 213-B				
Control	0	0	15,000,000	2.000
	3	465,000	3,500,000	1.368
	5	775,000	136,000	-0.043
	6	930,000	3,330	-1.654
	7	1,085,000	380	-2.596
	8	1,240,000	35	-3.632
Catalase	0	0	16,900,000	2.000
	3	465,000	6,800,000	1.605
	5	775,000	1,690,000	1.000
	6	930,000	710,000	0.623
	7	1,085,000	233,000	0.139
	8	1,240,000	59,000	-0.456
b) PA-3679				
Control	0	0	10,700,000	2.000
	5	775,000	61,500	-0.241
	6	930,000	8,100	-1.121
	7	1,085,000	145	-2.868
Catalase	0	0	11,600,000	2.000
	5	775,000	1,460,000	1.100
	6	930,000	410,000	0.548
	7	1,085,000	179,500	0.1895

*One rep unit is a dose of ionizing radiation capable of producing energy absorption of 93 ergs per gram of tissue.



Effect of catalase on the lethality of gamma radiation from cobalt-60 for anaerobic bacterial spores.

REFERENCES

Allen, A. O., (1954). The yields of free H and OH in the irradiation of water, Radiation Research, 1, 85-96.

Burnett, W. T., et al., (1951). Reduction of x-ray sensitivity of Escherichia coli B/r by sulfhydryl compounds, alcohols, glycols, and sodium hydrosulfite. Proc. Soc. Exp. Biol. and Med., 77, 636-638.

Curran, H. R., Evans, F. R., and Leviton, A., (1940). The sporicidal action of hydrogen peroxide and the use of crystalline catalase to dissipate residual peroxide. J. Bact., 40, 423-434.

Kempe, L. L., Graikoski, J. T., and Gillies, R. A., (1954). Gamma ray sterilization of canned meat previously inoculated with anaerobic bacterial spores. Applied Microbiol., 2, 330-332.

Hollaender, A., Stapleton, G. E., and Martin, F. L., (1951). X-ray sensitivity of E. coli as modified by oxygen tension. Nature, 167, 103-104.

Reed, J. M., Bohrer, C. W., and Cameron, E. J., (1951). Spore destruction rate studies on organisms of significance in the processing of canned foods. Food Research, 16, 383-408.