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UNITED STATES ATOMIC ENERGY COMMISSION

AECU-2886

INVESTIGATION OF GAMMA STERILIZATION

Research Report No. 7-53

By
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December 1953

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National Canners Association
Washington, D. C.

Technical Information Service, Oak Ridge, Tennessee

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Research Report
No. 7-53

Investigation of Gamma Sterilization

Summary

Results obtained in irradiation studies conducted between August, 1952 and December, 1953 are summarized as follows:

- (1) The amount of oxygen present during irradiation affects the survival of bacterial spores. In general, oxygen is toxic to spores during irradiation.
- (2) Irradiation at sub-sterilizing doses appears to decrease the resistance of surviving bacterial spores to later heat exposures.
- (3) Strong heat treatments prior to irradiation do not have a substantial effect upon the subsequent sensitivity of bacterial spores to irradiation.
- (4) The amount of aeration and the temperature during spore formation, prior to irradiation, have an effect upon the resistance of bacterial spores to gamma rays.
- (5) Variation of the hydrogen ion concentration of a suspending buffered phosphate solution within the pH range of 5 to 8 does not affect bacterial spore resistance to irradiation.
- (6) Spores of several species when exposed to gamma rays in mixed suspensions have the same or slightly higher resistance to irradiation than when they are exposed separately in pure suspension.
- (7) Spores irradiated in a dry (freeze-dried) state have a greater resistance to irradiation than when suspended in aqueous solution.

The program undertaken by this laboratory in early 1952 to study the effect of gamma rays upon microorganisms was outlined in Research Report No. 5-53, which presented the laboratory results up to August, 1952. This report covers the results of the project between August, 1952 and December, 1953.

The project was supported entirely by the National Canners Association up to July, 1953. We are indebted to the Atomic Energy Commission for a grant used to help us continue the study since that time.

The introduction and methods presented in Research Report No. 5-53 are applicable to the following work except where otherwise noted. All of the samples were exposed to gamma rays from Cobalt 60.

For ease in combining the earlier report and this report the same style and coding of experiments is continued.

Special acknowledgement is made for the generous assistance given to this project by personnel of the National Canners Association Research Laboratory other than the author.

(J) Determination of the Effect of the Amount of Oxygen Available to Spores during Irradiation upon Their Survival.

Spores of an aerobe (F.S. #1518) and an anaerobe (Clostridium botulinum No. 62-A) were each suspended in one of two solutions which were then sealed in glass vials under three different gaseous environments.

The two suspending liquids were either Sorenson's buffered phosphate (pH 6.8) or a 0.5% mixture of both ascorbic acid and thiourea in water (pH 6.8) - the latter mixture having a lower redox potential than the buffered phosphate. After this experiment was concluded, references were found which indicated that thiourea has a notable protective ability during irradiation upon the enzyme carboxypeptidase.* Since Burnett** et al, have shown a protective effect upon bacteria of another sulfur compound, $\text{Na}_2\text{S}_2\text{O}_3$, there may have been an uncontrolled variable in the liquid phase of this experiment.

The spores, suspended in either of the above substrates, were sealed in glass thermal death time vials under (a) atmospheric pressure, (b) 28 inches of vacuum, (c) nitrogen - after evacuating to 28 inches of vacuum.

Irradiation was at room temperature using Cobalt 60 source No. 15 which had a reported dosage rate of 220,000 R/hr.

As shown in Tables 1 and 2, (plotted in Graphs 1 and 2) exposure of the spores in a liquid of lowered redox potential appears to have a definite protective effect during irradiation. The protection afforded the aerobe, Flat Sour #1518, is somewhat greater than that evidenced with the anaerobe.

* Dale, Walter M., Symposium on Radiation Microbiology and Biochemistry, March, 1952, J. CELLULAR COMP. PHYSIOL., Supplement 1, 39, 39-55.

** Burnett, Jr., Morse, Burke, and Hollaender, May, 1952, J. BACT., 63, 5, 591.

The effect of changing the gaseous atmospheres above the liquid spore suspension is shown in Tables 3 and 4, and is plotted in Graphs 3 and 4. Here, again, the exclusion of air during irradiation allows a greater number of spores to survive. In this case, however, the greater protective effect is noted using the anaerobe, Cl. botulinum No. 62-A.

Table 1

Survival of Clostridium botulinum No. 62-A Spores
Irradiated in Various Liquid Substrates.

Exposure Mega- roentgens	Buffered Phosphate ⁽¹⁾		0.5% Ascorbic Acid-Thiourea ⁽¹⁾	
	Count	Log % Survival	Count	Log % Survival
0	4,200,000	2.000	2,700,000	2.000
0.2	3,800,000	1.956	2,800,000	2.016
0.4	2,100,000	1.694	2,000,000	1.873
0.6	480,000	1.058	1,300,000	1.682
0.8	120,000	0.456	370,000	1.137
1.0	36,000	-1.933	107,000	0.598
1.2	4,300 ^(*)	-1.010	23,400	-1.938
1.4	34	-4.908	3,700 ^(*)	-1.137
1.6	8	-4.280	702	-2.415
1.8	6	-4.155	56	-4.317

(1) Each count is the mean of 9 individual, irradiated vials.

(*) Recoveries below these levels are not plotted in Graph 1.

Table 2

Survival of Flat Sour No. 1518 Spores Irradiated in Various Liquid Substrates

Exposure Mega- roentgens	Buffered Phosphate ⁽¹⁾		0.5% Ascorbic Acid - Thiourea ⁽¹⁾	
	Count	Log % Survival	Count	Log % Survival
0	353,000	2.000	497,000	2.000
0.2	122,000	1.538	174,000	1.544
0.4	39,000	1.042	73,000	1.167
0.6	7,500	0.326	28,400	0.757
0.8	906	-1.409	10,170	0.311
1.0	97 ^(*)	-2.438	1,980 ^(*)	-1.600
1.2	16	-3.656	48	-3.985
1.4	1	-4.452	18	-3.559
1.6	0		0	
1.8	0		0	

(1) Each count is the mean of 9 individual, irradiated vials.

(*) Recoveries below these levels are not plotted in Graph 2.

Graph 1—Survival of spores of Cl. botulinum in menstrum of different redox potential.

Graph 2—Survival of spores of F.S. 1518 in menstrum of different redox potential.

Table 3

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Survival of Clostridium botulinum No. 62-A Spores
Irradiated under Various Gaseous Atmospheres.

<u>Exposure</u> Mega- roentgens	<u>Nitrogen</u> (#) (1)		<u>Vacuum</u> (1)		<u>Atmosphere</u> (1)	
	Count	Log % Survival	Count	Log % Survival	Count	Log % Survival
0	4,600,000	2.000	2,500,000	2.000	2,300,000	2.000
0.2	4,300,000	1.970	2,600,000	2.017	2,600,000	2.053
0.4	2,600,000	1.752	2,100,000	1.924	1,400,000	1.784
0.6	1,490,000	1.509	1,500,000	1.778	360,000	1.194
0.8	330,000	0.856	540,000	1.334	80,000	0.541
1.0	111,000	0.382	170,000	0.832	14,800	-1.809
1.2	15,700(*)	-1.536	41,800	0.223	8,500	-1.568
1.4	37	-4.905	7,500(*)	-1.477	391(*)	-2.230
1.6	14	-4.483	1,880	-2.876	108	-3.672
1.8	49	-3.027	58	-3.366	13	-4.752

(#) Nitrogen had an estimated oxygen content of 0.2%.

(1) Each count is the mean of 6 individual, irradiated vials.

(*) Recoveries below these levels are not plotted in Graph 3.

Table 4

Survival of Flat Sour No. 1518 Spores Irradiated under Various Gaseous Atmospheres.

Exposure Mega- roentgens	Nitrogen (#) (1)		Vacuum (1)		Atmosphere (1)	
	Count	Log % Survival	Count	Log % Survival	Count	Log % Survival
0	460,000	2.000	422,000	2.000	392,500	2.000
0.2	200,000	1.638	149,500	1.548	114,500	1.465
0.4	74,000	1.206	56,500	1.127	38,000	0.986
0.6	14,700	0.504	20,500	0.686	18,400	0.671
0.8	5,800	0.100	8,080	0.282	2,729	-1.842
1.0	808(*)	-1.244	1,129(*)	-1.427	283(*)	-2.858
1.2	50	-2.036	28	-3.822	18	-3.661
1.4	13	-3.451	10	-3.375	3	-4.883
1.6	11	-3.379	0		0	
1.8	0		0		0	

(#) Nitrogen had an estimated oxygen content of 0.2%.

(1) Each count is the mean of 6 individual, irradiated vials.

(*) Recoveries below these levels are not plotted in Graph 4.

Graphs 3 and 4—Survival of spores under different gaseous environments.

(K) Determination of the Effect of Partial Irradiation
on the Subsequent Heat Resistance of Surviving Spores.

Spores of three organisms (T.A. #3814, P.A. #3679 and B. coagulans #2253) in buffered phosphate were individually sealed in thermal death time vials. One-fourth of the tubes were not irradiated, while each of the remaining fourths received either 0.25, 0.60 or 1.00 mega-roentgens. Exposure was in Cobalt 60 sources No. 17 and No. 18 having reported dosage rates of 238,000 R/hr. and 237,000 R/hr. respectively.

After irradiation the suspensions of T.A. #3814 and P.A. #3679 were heated for varying time intervals at 240°F. The suspension of B. coagulans, being less heat resistant, was heated at 212°F.

The thermophilic anaerobe #3814 was cultured in Reynolds anaerobic medium (0.5% yeast extract, 0.1% sodium thioglycollate, 1.5% tryptone, and 1.5% agar) and incubated six days at 55°C.

The results shown in Table 5, and plotted in Graphs 5 and 6 show a definitely increased sensitivity of spores to heat after irradiation at sub-sterilizing levels. The data for P.A. #3679, while not extensive enough to plot, show the same trend as the other two spore suspensions.

Table 5

Resistance of Bacterial Spores to Heat after Receiving Sub-Sterilizing Doses
of Gamma Radiation

Time Heated (minutes)	T.A. #3814				P.A. #3679				B. coagulans 2253			
	240°F.				Exposure Temperature 240°F.				212°F.			
	Exposure in Mega-roentgens				Exposure in Mega-roentgens				Exposure in Mega-roentgens			
	0	0.25	0.60	1.00	0	0.25	0.60	1.00	0	0.25	0.60	1.00
0	4,600,000	270,000	15,000	53	500,000	140,000	33,000		6,300,000	99,000.	2,200	
1	360,000	6,200	83	3	70,000	5,300	0			64,000	1,210	
1.5			3	0			0					
2	40,000	240	2	0	24,000	220	0		5,300,000		690	
2.5						80						
3	11,000	16	0		3,000	0.33			4,500,000	37,000	490	
4	1,700	5										
5		2							3,300,000	20,000	340	
6	220	0.75			0				3,000,000	25,000	230	
9	5				0				1,730,000	6,300	110	
11									1,030,000	5,000		
13									390,000			
14.25									416,000			

LOG %
SURVIVAL

Graph 5—Survival of spores of T.A. 3814 heated after irradiation.

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Graph 6—Survival of spores of *B.coagulans* 2253 heated after irradiation.

(L) Determination of the Effect of Partial Heat Inactivation upon the Resistance of the Surviving Spores to Subsequent Gamma Radiation.

Spores of four organisms (F.S. #1518, T.A. #3814, P.A. #3679, and B. coagulans #2253) in buffered phosphate were sealed in thermal death time tubes. The organisms were then heated to kill an estimated 80% of the original population. Heating times of 10 minutes, 2.2 minutes and 1.0 minute at 240°F. were used respectively for F.S. #1518, T.A. #3814 and P.A. #3679. B. coagulans #2253 was heated for 2.2 minutes at 212°F.

After the heat treatment, the tubes were subjected to increasing dosages of gamma rays in Cobalt 60 sources No. 17 and No. 18 having reported dosage rates of 238,000 R/hr. and 237,000 R/hr. respectively. T.A. #3814 was cultured on Reynolds anaerobic medium. Table 6, and the same data plotted in Graphs 7 through 10, shows the resistance to gamma radiation of these spore suspensions heated prior to irradiation compared with composite data on the unheated suspensions.

It appears that, within experimental error, there is no difference in resistance of these spores to gamma rays induced by heat prior to irradiation.

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Table 6

Resistance of Bacterial Spores to Gamma Rays after Receiving a Sub-Sterilizing Heat Shock (80% estimated kill)

Exposure Mega- roentgens	T.A. #3814			P.A. #3679			F.S. #1518			B. coagulans #2253		
	Count	Log % Survival		Count	Log % Survival		Count	Log % Survival		Count	Log % Survival	
	Heated	Heated	Control	Heated	Heated	Control	Heated	Heated	Control	Heated	Heated	Control
0	250,000	2.000	2.000	36,100	2.000	2.000	97,300	2.000	2.000	670,000	2.000	2.000
0.2	30,120	1.079	1.257	2,440	0.829	1.376	28,500	1.465	1.279	84,400	1.097	0.714
0.3	13,460	0.730	0.844	4,270	1.072	1.490	23,300	1.378	0.948	15,970	0.377	-
0.5	2,270	-1.958	0.079	410	0.053	0.998	3,520	0.558	0.205	1,500	-1.350	-1.644
0.6	1,090	-1.640	-1.679	250	-1.840	0.693	485	-1.697	-1.864	110	-2.215	-2.397
0.7	290	-1.064	-1.288	320	-1.947	0.317	107	-1.037	-1.500	17	-4.403	-2.094
0.8	120	-2.681	-2.897	150	-1.618	-1.920	36	-2.568	-1.124	4	-5.769	-3.830
1.0	25	-2.000	-2.126	12	-2.521	-1.265	7	-3.857	-2.382	0.5		
1.2	3			4			0.2					
1.4	1			1.5								

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Graphs 7 and 8—Effect of preirradiation heating upon spore survival.

Graphs 9 and 10—Effect of preirradiation heating upon spore survival.

(M) Determination of the Effect of Growth Conditions during Spore Formation and Conditions during Irradiation upon Spore Survival during Irradiation.

Six spore suspensions were produced from a stock culture of B. coagulans #2253. Three of these were produced in Kolle flasks on the surface of a solid medium (Difco Thermoacidurans Agar) and three were produced in Erlenmeyer flasks of liquid medium (same composition as above but without agar) while being agitated on a shaking machine to provide maximum aeration.

Of these six spore suspensions, one in liquid and one on agar were produced at 28°C. A second pair was produced at 37°C. and a third at 55°C.

The six spore suspensions were concentrated by centrifugation and each re-suspended in buffered phosphate at pH 5, 7, or 8.

Exposure was in Cobalt 60 sources No. 16 and No. 18 having reported dosage rates of 236,000 R/hr. and 237,000 R/hr. respectively. The numbers of survivors after irradiation of these spore suspensions is given in Table 7. By pooling results from this Table and converting to percent survival the individual variables are analyzed in Tables 8, 9, and 10.

The results of this study indicate a definite effect caused by the temperature of production and the medium used while no effect appeared due to pH of the spore substrate during irradiation.

Table 7

Survival of Bacterial Spores Produced and Irradiated
under Various Conditions when Exposed to Gamma Rays.

Spore Production		Irrad. pH	Exposure in Mega-roentgens			
Temperature	Medium		0	0.2	0.5	0.8
			Total Count*			
28°C.	Agitated liquid	5	910,000	31,000	375	10
"	Solid	"	450,000	32,000	1,139	33
"	Agitated liquid	7	1,180,000	29,000	433	14
"	Solid	"	410,000	28,000	1,528	46
"	Agitated liquid	8	870,000	36,000	1,120	6
"	Solid	"	240,000	16,000	260	1
37°C.	Agitated liquid	5	850,000	27,000	309	3
"	Solid	"	83,000	2,500	17	8
"	Agitated liquid	7	730,000	19,000	164	4
"	Solid	"	87,000	1,500	41	14
"	Agitated liquid	8	700,000	3,200	445	21
"	Solid	"	59,000	1,500	18	1
55°C.	Agitated liquid	5	260,000	5,800	116	8
"	Solid	"	1,140,000	36,000	200	11
"	Agitated liquid	7	390,000	8,100	110	5
"	Solid	"	1,750,000	39,000	581	100
"	Agitated liquid	8	370,000	14,000	105	1
"	Solid	"	1,250,000	41,000	295	4

* Each count represents the mean of 4 irradiated vials.

Table 8

Effect of Temperature during Spore Production upon Survival during Irradiation.

Exposure Mega- roentgens	Temperature during Spore Production					
	(1) 28°C.	(2) 37°C.		(3) 55°C.		
		2/ 1		3/ 1		
0.2	5.0% survival	2.90% survival 0.58		2.76% survival 0.55		
0.5	0.163 "	0.037 " 0.23		0.029 " 0.18		
0.8	0.0053 "	0.00152 " 0.29		0.00147 " 0.28		

Each figure represents the mean of 24 irradiated vials.

Table 9

Effect of pH of Suspending Menstruum on Spore Exposed to Gamma Radiation.

Exposure Mega- roentgens	pH of buffered phosphate menstruum		
	5	7	8
0.2	3.7% survival	3.0% survival	3.5% survival
0.5	0.076 "	0.089 "	0.064 "
0.8	0.0028 "	0.0051 "	-

Each figure represents the mean of 24 irradiated vials.

Table 10

Effect of Aeration during Spore Production upon Survival during Irradiation.

<u>Exposure</u> Mega- roentgens	Method of Spore Production		$\frac{2}{I} \times 100$
	<u>Spores grown on solid agar surface (1)</u>	<u>Spores grown in agitated liquid medium (2)</u>	
0.2	4.0% survival	3.1% survival	77
0.5	0.105 "	0.048 "	46
0.8	0.00664 "	0.00125 "	19

Each figure represents the mean of 36 irradiated vials.

(N) Effect of Gamma Rays upon Mixed Spore Suspensions.

Spores of four organisms (F.S. #1518, T.A. #3814, Cl.botulinum No. 62-A, and Cl.nigrificans #3750) were suspended in buffered phosphate at pH 6.8. These suspensions were then sealed in thermal death time tubes as either pure suspensions or mixtures of the various pure suspensions. They were then subjected to increasing exposures of gamma radiation in Cobalt 60 sources No. 22 and No. 23 having reported dosage rates of 235,000 R/hr.

F.S. #1518 was cultured in petri plates of dextrose-tryptone agar. T.A. #3814 was cultured in shake tubes of medium composed of 1.5% agar, 0.5% yeast extract, 0.1% sodium thioglycollate and 1.5% tryptone with 500,000 p.p.m. of gentian violet. Cl.nigrificans was cultured in standard sulfite agar.

The code used for reporting the data is presented below with the mean original total population in each type of thermal death time tube as determined by recovery counts on the particular tubes.

<u>Code</u>		<u>Original Concentration</u>
A	<u>B. stearothermophilus</u> (F.S. #1518)	188,000/ml
B	<u>Cl. thermosaccharolyticum</u> (T.A. #3814)	700,000
C	<u>Cl. botulinum</u> No. 62-A	260,000
D	<u>Cl. nigrificans</u> #3750	68,000
ABC	Mixtures of the above	467,000
ACD	"	435,000
ABD	"	997,000
ABCD	"	1,250,000

The number of recovered spores is shown in Table 11 for each component of the mixtures. The total number of all spores recovered from pure and mixed suspensions is presented in Table 12.

The recovered numbers of a particular type of spore from pure or mixed suspensions are plotted on Graphs 11 through 14. The total numbers of all spores recovered from both pure and mixed suspensions are plotted in Graph 15.

The results plotted in Graphs 11, 12, 13 and 14 show a slight increased survival when a spore species is in a mixture during irradiation over that when it is in a pure suspension. In the case of F.S. #1518 there appears to be a definite protective effect in the presence of other spores.

When the total spore survival count, regardless of spore type is plotted, as in Graph 15, there seems to be a slight, protective effect when spores are mixed.

Table 11

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Total Number of Specific Spores Recovered from Mixed Suspensions Exposed to Gamma Rays

Spores Present*	Exposure Megaröntgens	Numbers of specific types of spores present*							
		A		B		C		D	
		Count	Log % Survival	Count	Log % Survival	Count	Log % Survival	Count	Log % Survival
ABC	0	39,000	2.000	150,000	2.000	260,000	2.000		
	0.2	48,000	2.090	110,000	1.865	250,000	1.983		
	0.6	4,500	1.061	5,800	0.585	49,000	1.274		
	0.8	776	0.297	2,300	0.185	22,600	0.939		
	1.0	12	-2.487	310	-1.314	5,500	0.324		
	1.2	21	-2.731	8	-	310	-1.075		
ACD	0	81,000	2.000			280,000	2.000	74,000	2.000
	0.2	17,000	1.320			122,000	1.639	3,500	0.674
	0.6	3,190	0.594			37,000	1.120	350	-1.674
	0.8	900	0.045			9,700	0.538	108	-2.173
	1.0	212	-1.417			2,780	-1.996	42	-3.754
	1.2	23	-2.452			2,160	-1.887	1	-
ABD	0	67,000	2.000	810,000	2.000			120,000	2.000
	0.2	40,000	1.776	111,000	1.137			21,000	1.243
	0.6	3,200	0.678	4,400	-1.735			1,300	0.033
	0.8	1,760	0.418	2,460	-1.481			140	-1.068
	1.0	318	-1.675	558	-2.838			30	-2.398
	1.2	54	-2.906	12	-3.170			4	-
ABCD	0	88,000	2.000	440,000	2.000	610,000	2.000	110,000	2.000
	0.2	143,000	2.209	92,000	1.320	350,000	1.759	13,000	1.072
	0.6	14,300	1.209	4,400	0.000	87,000	1.152	700	-1.803
	0.8	6,200	0.848	1,400	-1.502	21,900	0.555	110	-1.000
	1.0	5,000	0.754	567	-1.110	8,340	0.137	30	-2.436
	1.2	10	-2.531	17	-3.586	948	-1.190	0	-

* see p. 24 for code interpretation

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Table 12

Total Number of Spores Recovered from Pure and Mixed Suspensions Exposed to Gamma Rays

Exposure Mega-roentgens	Types of Spores in Irradiation Vial *															
	A		B		C		D		ABC		ACD		ABD		ABCD	
	Count	Log %	Count	Log %	Count	Log %	Count	Log %	Count	Log %	Count	Log %	Count	Log %	Count	Log %
	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.	Surv.
0	188,000	2.000	700,000	2.000	260,000	2.000	68,000	2.000	449,000	2.000	435,000	2.000	997,000	2.000	1,250,000	2.000
0.2	52,000	1.441	56,000	0.903	190,000	1.863	7,200	1.021	408,000	1.958	142,000	1.513	172,000	1.238	598,000	1.679
0.6	690	-1.566	4,200	1.778	21,000	0.907	190	1.445	59,300	1.121	40,500	0.969	8,900	-1.951	106,000	0.928
0.8	66	-2.545	390	-2.746	4,500	0.238	124	-1.260	25,670	0.757	10,700	0.389	4,360	-1.640	29,600	0.577
1.0	3	-3.201	180	-2.410	1,200	1.664	14	-2.312	5,822	0.113	3,034	-1.843	906	-2.958	13,900	0.045
1.2	0	-	25	-3.510	90	-2.540	4	-3.769	339	-2.878	2,184	-1.700	70	-3.846	975	-2.892

* see page 24 for code interpretation

Graph 11 — Survival of spores of F.S. 1518 in mixed and pure suspensions.

Graph 12—Survival of spores of T.A. 3814 in mixed and pure suspensions.

Graph 13—Survival of spores of *Cl. nigrificans* 3750 in mixed and pure suspensions.

Graph 14—Survival of *Cl. botulinum* 62A in mixed and pure suspensions.

Graph 15—Survival of spores in mixed and pure suspension.

(0) Effect of Irradiation of Spores in the Dry and Wet State upon Survival.

Spores of C1.botulinum No. 62-A were suspended in an aqueous solution of 0.5% ascorbic acid, 0.5% thiourea and 2.0% dextrin at final pH of 6.8. Half milliliter amounts of the suspension were filled into thermal death time vials, and all vials were freeze-dried at 100 microns of pressure or below for 18 hours. Half of the freeze-dried tubes were made up to original volume with water and sealed under 28 inches of vacuum while the remaining tubes were sealed directly after freeze-drying while still in the dry state.

Both sets of vials were subjected to increasing amounts of radiation in Cobalt 60 sources No. 16 and No. 22 having reported dosage rates of 250,000 R/hr. and 275,000 R/hr. respectively. Numbers of recovered spores per vial are given in Table 13 and plotted in Graph 16.

The irradiation resistance of spores in the dry state under the conditions of this experiment was greater than that in the wet state.

Table 13

Recovery of Spores of Clostridium botulinum Irradiated
in "Dry" and "Wet" Environments.

<u>Exposure</u> <u>Mega-</u> <u>roentgens</u>	<u>"Dry" Spores</u>		<u>"Wet" Spores</u>	
	<u>Count</u>	<u>Log % Survivors</u>	<u>Count</u>	<u>Log % Survivors</u>
0	6,200,000	2.000	3,000,000	2.000
0.2	3,000,000	1.684	1,700,000	1.753
0.4	3,800,000	1.787	1,300,000	1.636
0.5	3,800,000	1.787	1,600,000	1.727
0.6	4,100,000	1.820	1,300,000	1.637
0.7	3,400,000	1.736	1,000,000	1.522
0.8	2,500,000	1.605	640,000	1.328
0.9	2,800,000	1.654	350,000	1.065
1.0	2,300,000	1.568	270,000	0.954
1.2	390,000	0.799	120,000	0.602
1.4	250,000	0.605	44,000	0.164
1.5	390,000	0.798	27,000	-1.954
1.6	340,000	0.739	50,000	0.220

Graph 16—Survival of spores of *Cl. botulinum* 62A in environments of different water content.

Conclusion

2 The results reported emphasize the importance in gamma ray sterilization studies of thoroughly investigating the effect of conditions during spore production and spore irradiation. It is shown that variations in spore production temperature and variations in gaseous environment during spore production or irradiation may increase or decrease the sterilization requirement for a particular type of organism.

3 Definition of a sterilizing gamma radiation dosage with respect to spore forming organisms should assume an environment affording maximum protection. Such environmental conditions require further investigation.

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