1
UNITED STATES ATOMIC ENERGY COMMISSION

AECU-2885

INVESTIGATION OF GAMMA STERILIZATION

Research Report No. 5-53

By
B. H. Morgan

June 1953

Washington Research Laboratories
National Canners Association
Washington, D. C.

Technical Information Service, Oak Ridge, Tennessee

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Subject Category, BIOLOGY.

Work performed under Contract No. AT(30-1)-1567.

Permission of the author or originating installation is required for further reproduction of this report.

This report has been reproduced with minimum alteration directly from manuscript provided the Technical Information Service in an effort to expedite availability of the information contained herein.

Research Report
No. 5-53

Investigation of Gamma Sterilization
B.H. Morgan

Summary

Spores of thermophilic anaerobes, flat sours, putrefactive anaerobes, and *Clostridium botulinum* were subjected to gamma radiation from cobalt 60 and tantalum 182. As a result of these preliminary studies concluded in August, 1952, the following conclusions have been drawn:

- (1) Spore destruction by irradiation for practical purposes appears to be an exponential function of the amount of irradiation received.
- (2) Under the conditions of our irradiation the location of the spore-containing vials within the source, does not affect the lethality of the irradiation upon this spore population.
- (3) Culturing irradiated spore suspensions, stored at 4°C. for up to 8 weeks after irradiation, does not affect the number of viable spores present.
- (4) The intensity of the irradiation source, per unit of time, does not change the total dose needed to sterilize a spore suspension.
- (5) Spores of *Clostridium botulinum* are the most irradiation resistant spores of those tested using our experimental conditions.
- (6) The percent of the original spore population killed, short of sterility with a given dose of gamma rays is independent of the original population. The dose needed to achieve sterility is dependent upon the original population.

Introduction

The search for methods of sterilization of foods so as to overcome changes in flavor, texture and color caused by customary sterilization procedures, has created an interest in "cold" sterilization methods by the use of radioactive isotopes.

This interest has been furthered by the fact that the Atomic Energy Commission is continuously storing away as by-products of plutonium production, vast quantities of secondary irradiation sources, or fission products, capable of sterilizing materials exposed to their irradiation. To evaluate the practicability of the use of these atomic fission products by the canning industry as a means of sterilizing canned foods, a program has been undertaken in conjunction with the Brookhaven National Laboratory of the Atomic Energy Commission, and the results of the first six months of this project are reported in the following pages.

Earlier work in other laboratories on sterilization using different types of radiation as sterilizing agents, included the use of infra-red rays, ultra violet light, X-rays, and cathode rays. Infra-red rays are bactericidal only through the production of heat, and ultra violet light is effective as a sterilizing agent mainly upon surface areas. Beams of photons and electrons, such as X-rays and cathode rays, as a means of killing microorganisms have been studied in the Department of Food Technology in the Massachusetts Institute of Technology. These studies have shown that materials may be sterilized without an appreciable increase in temperature.

In general, the results of these studies have indicated that man-made or artificial X-rays are produced by allowing a stream of high energy electrons to impinge upon a target such as gold or tungsten. When these electrons strike a target much of the energy goes into wasted heat, while about 5% of the energy is released by an internal rearrangement of the atom,

as X-rays or photons. Similar photons which occur in nature are referred to as gamma rays.

Both X-rays and gamma rays have exactly the same properties and are similar except that the two arise from different sights within the atom. Their penetrating power is excellent in that they can pass through 4 to 6 inches of water before losing one-half of their energy. The potential use of X-rays is limited, however, due to the 95% loss of energy when the electron beam strikes the target in the production of X-rays. This deterrent is not present in the use of gamma rays from atomic fission products.

A second group of radiations is typified by the man-made cathode rays. This is an artificially produced stream of electrons without the introduction of the intermediate target used to produce X-rays. The identical, naturally occurring particle, found in the disintegration of fission products is the beta particle.

These latter two beams of electrons have identical properties. They both are high energy products with relatively slight penetrating properties. The strongest beta particle will lose the majority of its energy in passing through 15/100 inch of water.

Thus, there are available in general two types of irradiation, X-rays or gamma rays, and cathode rays or beta particles. For commercial utilization as means for sterilizing foods, the cathode rays, or beta particles, are limited by their low penetrating power and the X-ray by the economics of losing 95% of the production energy. From a practical standpoint, the naturally occurring gamma rays hold the most promise. It has been these gamma rays, originating from atomic disintegrations, that have been used in our studies.

The fission products from which these gamma rays will arise during the industrial use of this type of irradiation and sterilization are not available, nor will they be in the near future. If this means of sterili-

zation were to be proved practical it is estimated, by the Atomic Energy Commission, that these fission products would not be obtainable for large scale industrial use for the next ten years. However, when they do become available they will originate from the material resulting from the concentration of the liquid by-product of plutonium production and will be in a form that will give forth both gamma and beta rays. In the meantime, for experimental purposes, mock sources have been supplied by the Atomic Energy Commission which simulate, through the production of gamma radiation, the sterilizing efficiency of fission products. It is these mock sources, namely, cobalt 60 and tantalum 182, that have been used as sources in this work. Since this means of sterilization is a new concept in the preservation of foods the technical aspects of the production of the various types of radiation are taken up in a notation in the Appendix.

It has been shown in previous work in other laboratories that sterilization by irradiation involves not only the lethality to micro-biological life, but also an inactivation of the normal food enzymes. Both the work at the University of Michigan and that at the Massachusetts Institute of Technology have indicated that 4 to 10 times the sterilizing dose or more is needed to inactivate the enzymes systems found naturally in foods. Previous work has also indicated that certain of the vitamins are destroyed by irradiation at room temperature, although this effect has been shown to be decreased when the temperature of irradiation is lowered. Other important considerations involved in the sterilization by irradiation are possible changes in color and texture and the production of substances toxic to humans. To evaluate these numerous varying factors would involve an extensive experimental program. Consequently, we have restricted our initial studies to ²/determine the sterilizing ability of gamma rays on representatives of the micro-biological populations found involved in food spoilage and food poisoning. ²/₃

Method

Spore suspensions were prepared using the methods recommended in the N.C.A. bulletins for various types of organisms. After centrifugation and re-suspension in distilled water, the suspensions were diluted to 1:10 prior to use, with either buffered phosphate ($M/5 \text{ KH}_2\text{PO}_4 - M/5 \text{ NaOH}$) pH 7.0 or pea juice (pureed peas. diluted 1:10, boiled 5 mins. and filtered.) pH 6.95. One milliliter of the spore suspension was placed in thermal death time pyrex tubes (9 mm O.D. x 70 mm long) and the tubes were heat sealed so that they had a final length of 2-3/4 to 3 inches. These tubes were then coded and assembled into bundles. The bundles contained a core of 4 tubes, 3 tubes of spore suspensions, and 1 dosimeter tube, and an outer shell of 8 tubes containing spore suspensions. The bundles were kept refrigerated (4°C. to 8°C.) at all times except during irradiation at room temperature ($23 - 24^\circ\text{C.}$).

Irradiation at the Brookhaven National Laboratory consisted of placing 3 bundles at a time, one above the other, in an aluminum cylinder (of approximately 1.5 x 10.5 inches).

The cylinder was then lowered into an active cobalt 60 or tantalum 182 source for gamma irradiation. At varying time intervals individual bundles were removed, and placed in a $4^\circ - 8^\circ\text{C.}$ icebox. After all samples of Phase I or of Phase II had been completed all samples were returned, at icebox temperature, to the Washington Laboratory for bacteriological analysis.

Methods of counting the different organism are outlined below:

<u>Organism</u>	<u>Media</u>	<u>Incubation Minimum</u>
F.S. #1518	B.C.P. glucose tryptone agar	48 hours at 55°C.
F.S. #1503	B.C.P. glucose tryptone agar	48 hours at 55°C.
T.A. #3814	Tryptone-glucose-starch-thioglycolate agar, stratified	1 week at 55°C.
T.A. #2285	"	1 week at 55°C.
P.A. #3679	"	4 weeks at 37°C.
<u>Cl.botulinum</u> #62-A	Reynolds bicarbonate, stratified	6 weeks at 30°C.
<u>Cl.botulinum</u> #213-B	"	3 weeks at 30°C.

Ten separate experiments reported here were performed in two separate series of irradiations.

The dosimetry was done by the staff of the Brookhaven Laboratory using a ceric sulfate reduction method.

(A) Determination of Destruction Rates and Requirements
for Total Destruction of Several Typical Food
Poisoning and Food Spoilage Organisms.

Spore suspensions of four organisms were exposed to irradiation with a cobalt 60 source, having an average dosage rate of 193,000 roentgens per hour. The range of the total dosage to which the spore suspensions were exposed was from 55,000 to 1,760,000 R which covered an exposure time between 17 min. and 9 hours and 8 min. The destruction rate for these spores when suspended in buffered phosphate is shown as a survivor curve in Graph I. The destruction rate of these spores in 1:10 pea puree is shown in a like manner in Graph II.

There appears to have been no difference in number of recovered viable organisms when the spores were suspended in buffered phosphate or compared to suspension in a 1:10 pea puree. The difference in survivor curves in the two substrates with P.A. 3679 was probably due to the limited population of the sample tubes.

The destruction of the spores of all four organisms by gamma irradiation appears to be an exponential function of the amount of irradiation received as determined in this and later experiments. However, there are indications that at low dosages of gamma rays (less than 100,000 R) the survivor curve tends toward a sigmoidal curve. For the purposes of process determination the curve can be considered a straight line.

The resistance of the four types of spore suspensions in phosphate to gamma irradiation bore little relationship to the resistance of these same spore suspensions to elevated temperatures. Listed below, placed in decreasing order of resistance, are the test organisms relative resistance to the two modes of sterilization:

Heat	Irradiation
F.S. 1518	Cl.botulinum 62-A
P.A. 3679	P.A. 3679
T.A. 3814	T.A. 3814 and F.S. 1518
Cl.bot. 62-A	

Tubes of these suspensions were held after irradiation at 4°C. for several weeks and counted again. These results are reported in the next experiment.

Graph I—Survival of spores irradiated in phosphate.

Graph II—Survival of spores irradiated in pea juice.

(B) The Study of the Effect of Location in the Source Upon Lethality and Determination of the Delayed Lethality During Storage Period Subsequent to Irradiation.

Spore suspensions of F.S. 1518 and P.A. 3679 in phosphate were subjected to increasing amounts of gamma irradiation from a cobalt 60 source. Tubes from the core and outside layer of each bundle were cultured soon after irradiation and after storage at 4°C. for 4 and 8 weeks.

From the data in Tables 1 and 2 the location of the tube within the individual bundle did not affect the total dosage needed to reduce the spore population within the tubes.

There was no noticeable change in these results after storage for 4 or 8 weeks.

To further evaluate storage of irradiated spore suspensions irradiated tubes from Experiments A through G were held at 4°C. for varying lengths of time before culturing. The original and delayed counts for these tests are given in Tables 1 through 14.

From these data, within the experimental error caused by using a limited number of tubes, there appears to be no change in the recoverable number of spores either immediately after irradiation or up to 2 months later when stored at 4°C.

TABLE 1

Effect of varying location in bundle during irradiation
(F.S. #1518 in phosphate)

	Before		4 Weeks		8 Weeks
	Outside	Inside	Outside	Inside	Outside
Control	44,000,000	44,000,000			
Kiloroentgens					
55	11,900,000	14,200,000	26,000,000	18,000,000	14,500,000
110	11,200,000	9,400,000	14,200,000	15,900,000	18,000,000
165	6,200,000	6,700,000	10,900,000	10,600,000	8,800,000
441	1,350,000	1,400,000	1,170,000	1,170,000	2,800,000
882	21,000	19,500	20,000	20,000	25,000
1,100	540	1,730	400	800	300
1,330	300	160	310	300	640
1,540	80	13	10	10	38
1,760	1	13	0	0	0

TABLE 2

Effect of varying location in bundle during irradiation
(P.A. #3679 in phosphate)

	Before		4 Weeks		8 Weeks
	Outside	Inside	Outside	Inside	Outside
Control	15,000,000				
Kiloroentgens					
55	5,000,000	8,000,000	9,300,000	9,300,000	5,100,000
110	4,000,000	6,000,000	10,100,000	9,300,000	4,000,000
165	3,000,000	5,000,000	6,300,000	9,000,000	3,200,000
441	1,300,000	4,000,000	3,430,000	3,900,000	2,000,000
882	170,000	170,000	243,000	230,000	190,000
1,100	4,000	510,000	15,300	22,700	5,000
1,330	1,500	1,500	4,167	3,300	2,800
1,540	40	1,400	249	-	1,300
1,760	60	1	10	-	3

TABLE 3

Effect of storage (4°C.) after irradiation upon surviving spores
(F.S. #1518 in phosphate)

Control	Before	6 Weeks
	<u>30,000,000</u>	
Kiloroentgens		
26	17,000,000	32,000,000
52	16,000,000	17,500,000
78	15,000,000	22,000,000
208	9,200,000	6,000,000
416	1,130,000	1,220,000
520	75,000	150,000
624	1,164	110,000
728	1,700	90,000
832	67	1,800

TABLE 4

Effect of storage (4°C.) after irradiation upon surviving spores
(P.A. #3679 in phosphate)
(Irradiated with tantalum 182)

Control	Before	4 Weeks	6 Weeks
	<u>15,000,000</u>		
Kiloroentgens			
26	10,000,000	9,000,000	3,400,000
52	3,500,000	3,900,000	1,900,000
78	3,100,000	3,900,000	1,800,000
208	2,500,000	2,600,000	1,900,000
416	1,600,000	1,400,000	1,400,000
520	800,000	700,000	1,700,000
624	750,000	600,000	2,800,000
728	410,000	340,000	370,000
832	90,000	55,000	190,000

TABLE 5

Effect of storage (4°C.) after irradiation upon surviving spores
(F.S. #1518 in phosphate)

Count Before and After Storage at 4°C.

Control	Before	6 Weeks
	30,000,000	29,000,000
Kiloroentgens		
55	26,000,000	23,000,000
110	10,100,000	12,100,000
165	10,900,000	8,400,000
441	1,910,000	3,000,000
882	18,000	14,000
1,100	80	50
1,330	33	450
1,540	2	78
1,760	0	0

TABLE 6

Effect of storage (4°C.) after irradiation upon surviving spores
(F.S. #1518 in 1:10 pea puree)

Control	Before	6 Weeks
	44,000,000	
Kiloroentgens		
55	15,400,000	16,000,000
110	12,700,000	17,000,000
165	6,500,000	7,800,000
441	1,500,000	1,600,000
882	460,000	30,000
1,100	1,800	3,400
1,330	520	450
1,540	110	74
1,760	0	0

TABLE 7

Effect of storage (4°C.) after irradiation upon surviving spores
 (F.S. #1518 in phosphate)
 (Heated to 240°F. for 16.5 min. prior to irradiation)

Control	<u>Before Irradiation</u>	<u>After 7 Weeks at 4°C.</u>
	5,800,000	
Kiloroentgens		
55	5,700,000	7,300,000
110	4,600,000	5,300,000
165	3,000,000	1,500,000
441	580,000	380,000
882	8,000	5,000
1,100	69	90
1,330	80	70
1,540	4	12
1,760	0	2

TABLE 8

Effect of storage (4°C.) after irradiation upon surviving spores
(F.S. #1518 in phosphate)
(Irradiated prior to being heated to 240°F.)

<u>Min. Held at 240°F.</u>	<u>Irradiated</u>		<u>Not Irradiated</u>
	<u>Before</u>	<u>7 Weeks at 4°C.</u>	<u>Before</u>
0	14,600,000	15,000,000	15,900,000
15	4,200,000	3,100,000	4,800,000
30			1,930,000
40	710,000	160,000	
45			274,000
60	20,000	27,000	23,500
75			1,940
80	1,700	1,200	
90	240	350	240
95			569
100	4	10	136
105	0		43
110	0	4	12
115			5
120			1
130			0
140			0
150	0		

TABLE 9

Effect of storage (4°C.) after irradiation upon surviving spores
(P.A. #3679 in phosphate)

Control	<u>Before</u> 15,000,000	<u>After 6 Weeks</u>
Kiloroentgens		
55	4,400,000	3,400,000
110	3,600,000	2,900,000
165	3,000,000	2,500,000
441	2,500,000	1,700,000
882	120,000	90,000
1,100	33,000	1,000
1,330	30	16,000
1,540	20	64
1,760	1	1

TABLE 10

Effect of storage (4°C.) after irradiation upon surviving spores
(P.A. #3679 in 1:10 pea puree)

Control	<u>Before</u> 11,300,000	<u>After 3 Weeks</u>
Kiloroentgens		
55	3,650,000	4,900,000
110	2,700,000	5,200,000
165	3,800,000	4,200,000
441	3,000,000	2,800,000
882	130,000	76,000
1,100	120,000	16,000
1,330	12,000	2,800
1,540	10	300
1,760	0	3

TABLE 11

Effect of storage (4°C.) after irradiation upon surviving spores
(Cl. botulinum 62-A in phosphate)
 (Exposed to tantalum 182)

Control	<u>Before</u>	<u>After 6 Weeks</u>
	87,000,000	
Kiloroentgens		
26	80,000,000	46,700,000
52	93,000,000	52,000,000
78	90,000,000	28,300,000
208	67,000,000	46,000,000
416	21,000,000	36,000,000
520	10,000,000	17,300,000
624	13,700,000	25,700,000
728	8,700,000	14,300,000
832	3,700,000	-

TABLE 12

Effect of storage (4°C.) after irradiation upon surviving spores
(Cl. botulinum 62-A in phosphate)

Count Before and After Storage at 4°C.

Control	<u>Before</u>	<u>6 Weeks</u>
	87,000,000	
Kiloroentgens		
55	61,300,000	88,000,000
110	83,700,000	92,000,000
165	70,700,000	54,700,000
441	40,000,000	50,700,000
882	5,300,000	4,400,000
1,100	67,000	-
1,330	330,000	-
1,540	170,000	-
1,760	147	267

TABLE 13

Effect of storage (4°C.) after irradiation upon surviving spores
(Cl. botulinum 62-A in 1:10 pea puree)

Control	Before 125,000,000	6 Weeks
Kiloroentgens		
55	93,000,000	60,000,000
110	80,000,000	49,000,000
165	77,000,000	47,300,000
441	30,000,000	41,300,000
882	2,000,000	2,000,000
1,100	47,000	127,000
1,330	47,000	47,000
1,540	7,000	2,700
1,760	240	60

TABLE 14

Effect of storage (4°C.) after irradiation upon surviving spores
(T.A. #3814 in 1:10 pea puree)

Control	Before 5,100,000	6 Weeks 3,600,000
Kiloroentgens		
55	2,650,000	2,900,000
110	860,000	1,200,000
165	450,000	480,000
441	23,000	57,000
882	1,480	1,800
1,100	130	160
1,330	73	230
1,540	10	11
1,760	1	1

(C) Determination of the Destruction Rate and Requirement for Total Destruction of Several Typical Food Poisoning and Food Spoilage Organisms Employing an Irradiation Source of Lower Intensity than Cobalt 60.

In the previous exposures the source of gamma irradiation was cobalt 60 with an approximate dosage rate of 193,000 R/hr. In this run the source used, however, was of a lower intensity and was caused by the disintegration of another element, tantalum 182. This source has an approximate dosage rate of 39,000 R/hr.

Spore suspensions of F.S. 1518, T.A. 3814, P.A. 3679 and Cl.botulinum 62-A were exposed to increasing levels of irradiation from both cobalt 60 and tantalum 182. The percent of the spores viable after the different levels of irradiation are tabulated in Table 15.

These data show a slightly greater lethality in some cases when the spores had been exposed to tantalum 182. However, accepting the limits of the method of dosimetry and the number of tubes involved the source of gamma rays appears to have no effect on the lethality of those rays.

TABLE 15

Survival of spores exposed to gamma rays from
the decay of cobalt 60 and tantalum 182

Orig. Spore Conc.	1518		3814		3679		62-A	
	Cobalt	Tantalum	Cobalt	Tantalum	Cobalt	Tantalum	Cobalt	Tantalum
	30,000,000		2,650,000		15,000,000		87,000,000	
Roentgens	% Survival		% Survival		% Survival		% Survival	
26,000		56.66		83.02		66.6		91.95
52,000		53.33		48.68		23.33		100
55,000	86.6		71.7		29.33		70.45	
78,000		50.00		29.43		20.66		100
110,000	33.7		33.6		24.00		96.20	
165,000	36.3		17.7		20.00		81.26	
208,000		30.66		1.132		16.66		77.01
416,000		3.77		0.755		10.66		24.13
441,000	6.37		1.47		16.66		32.30	
520,000		0.25		0.109		5.33		11.49
624,000		0.0039		0.566		5.00		13.44
728,000		0.0057		0.0868		2.73		10.00
832,000		0.00022		0.0162		0.6		2.30
882,000	0.06		0.0185		0.80		6.09	
1,100,000	0.00027		0.00113		0.22		0.077	
1,330,000	0.00011		0.000717		0.0002		0.345	
1,540,000	0.000007		0.000226		0.00013		0.015	
1,760,000	0		0		0.000007		0.000168	

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

Spore suspensions of Cl.botulinum 62-A, and 213-B as well as F.S. 1518 in phosphate were irradiated to a total dosage of 26,000 R using a cobalt 60 source. This source had an average dosage rate of 193,000 R/hr. After irradiation the spore suspensions were held at 240°F. and the resulting survival curves compared to similar spore suspensions held at 240°F. but not previously irradiated.

Due to the sensitivity of Cl.botulinum to 240°F. only a limited number of points were available to plot a curve. In the case of both strains of this organism, however, those spores subjected to irradiation prior to heating had a lower number of surviving spores. With F.S. 1518 the two curves were identical through 90 mins. exposure to 240°F. After that, those spores which had been irradiated show less resistance as shown by the irradiated tubes being sterile after 105, 110, 120, and 130 while viable spores were recovered from the non-irradiated suspension through 120 mins. with sterility after 130 mins.

From these limited data available in this experiment there appears to be a slightly greater sensitivity of spores to heat (240°F.) after irradiation when compared to non-irradiated spores. This study is continuing to verify these early results.

(E) Determination of the Effect of a Partial Heat Inactivation Upon the Resistance of the Surviving Spores to Subsequent Gamma Radiation.

Spore suspensions of Cl.botulinum 62-A and F.S. 1518 were heated to a calculated 80% destruction by heating at 240°F. prior to irradiation. After heating the suspensions were subjected to increasing total dosages of gamma rays.

With spore suspensions of the thermophilic aerobic flat sour 1518 heating prior to irradiation endows some form of protection to the spores against irradiation, although this protection is slight. However, with the mesophilic anaerobic Cl.botulinum 62-A this reverse is true. That is the spore suspensions heated prior to irradiation appear to be more sensitive to irradiation.

These results prevent a definite conclusion other than to indicate further work. This study is continuing.

(F) Determination of Irradiation Needed to Sterilize Cl.botulinum 62-A

An attempt was made to achieve total sterility with a suspension of spores of Cl.botulinum 62-A, the most resistant organism used in Experiment A. However, due to an error in dosimetry the maximum requested irradiation was not achieved.

Table 16 shows the percent reduction of the population in four experiments using a spore concentration between 62 million and 125 million spores per milliliter.

One million roentgens appears to be the dosage needed to reduce the population by 99% when the spores are suspended in buffered phosphate. With original spore concentrations in the order of 60 to 80 million spores/ml. over 2,250,000 roentgens are needed to sterilize the suspension.

TABLE 16

Resistance of Clostridium botulinum 62-A to gamma radiation

	Exp. I	Exp. VIII	Exp. IV	Exp. II
	Cobalt 60 PO ₄	Cobalt 60 PO ₄	Tantalum 182 PO ₄	Cobalt 60 1:10 Pea Puree
Orig. Conc. -	87,000,000 org/cc	61,700,000 org/cc	87,000,000 org/cc	125,000,000 org/cc
	% Survival	% Survival	% Survival	% Survival
Roentgens				
26,000			91.95	
52,000			100.00	
55,000	70.45			74.40
78,000			100.00	
110,000	96.20			64.00
165,000	81.26			61.60
208,000			77.01	
416,000			24.13	
441,000	32.30			24.00
520,000			11.49	
624,000			13.44	
728,000			10.00	
832,000			2.298	
882,000	6.09			1.6
961,000		6.110		
1,100,000	0.077			0.0824
1,330,000	0.345			0.0376
1,540,000	0.0149			0.00560
1,600,000		0.0438		
1,760,000	0.000168			0.000192
1,810,000		0.00178		
2,030,000		0.000225		
2,130,000		0.000187		
2,340,000		0.0000113		

(G) Determination of the Effect of the Original Concentration of Microorganisms and the Concentration of the Suspending Food on the Roentgen Dosage Needed to Sterilize

Using the thermophilic flat sour 1518 (B. sterothermophilus) as a test organism, and pureed peas as a suspending food, three concentrations of organisms were made up in two different concentrations of pea puree. These spore suspensions were then subjected to increasing amounts of irradiation. Tables 17 and 18 show both the number of organisms recovered and the percent recovered after irradiation.

It is apparent that the greater concentration of pea puree endowed some form of protection to the spores allowing a greater recovery after irradiation. Also, a greater total dosage, measured in roentgens, is needed to achieve sterility when the original spore concentration is higher. This is especially true in the denser substrate.

The total percent reduction of the population short of sterility attained with a given dosage is, however, independent of the original concentration of spores. Thus, in the 1:10 puree using a dose of 213,000 R and varying the population between 33 million, 350,000 and 7,400, the percent survivors ranged from 21.42 to 28.48. As the number of organisms present decreases the percentage-wise importance of each organism increases so that as sterility approaches this relationship is not always obvious.

This is shown in Graph III where the survival curves are parallel with different original populations of spores.

SPORE
CONCENTRATION.

Graph III—Survival of spores of flat sour 1518 with varied original spore concentrations.

TABLE 17

Number of Surviving Spores After Irradiation of Three Population Levels in Two Concentrations of Pea Puree

Kilorontgens	<u>33,000,000</u>	<u>350,000</u>	<u>7,400</u>
213	9,400,000	75,000	1,800
426	2,700,000	6,400	89
641	115,000	180	10
1,070	13,000	17	1
1,270	1,210	2	1
1,500	99	0	0
1,760	1	0	0
1,890	6	0	0
2,130	0	0	0
	<u>66,000,000</u>	<u>930,000</u>	<u>20,500</u>
213	-	360,000	7,100
426	17,000,000	108,000	3,600
641	-	12,400	720
1,070	320,000	4,770	53
1,270	52,000	340	7
1,500	1,000	27	1
1,760	350	5	0
1,890	111	4	0
2,130	12	0	0

TABLE 18

Percent of Surviving Spores After Irradiation of Three Population Levels in Two Concentrations of Pea Puree

	<u>33,000,000</u>	<u>350,000</u>	<u>7,400</u>	
	28.48	21.42	24.32	
	8.18	1.83	1.20	
	0.348	0.05	0.135	
	0.0394	0.005	0.0135	1:10
	0.0037	0.0006	0.0135	Puree
	0.0003	0	0	
	0.000003	0	0	
	0.000002	0	0	
	0	0	0	
	<u>66,000,000</u>	<u>930,000</u>	<u>20,500</u>	
	-	38.7	34.63	
	25.76	11.6	17.56	
	-	1.33	3.51	
	0.485	0.513	0.258	Straight
	0.079	0.037	0.034	Puree
	0.0015	0.003	0.0049	
	0.0005	0.0005	0	
	0.00017	0.0004	0	
	0.00002	0	0	

(H) Determination of the Effect of Dosage Rate Used
for Irradiating Spores of Aerobic and Anaerobic
Thermophilic Bacteria Upon the Destruction Rate.

To determine the effect of rate of irradiation with gamma rays upon the bacterial spore suspensions three different sources were used: cobalt 60, tantalum 182 with a high rate, tantalum 182 with a low rate. The spore suspensions irradiated consisted of two thermophilic anaerobes and two thermophile aerobes.

The results of this exposure are presented in Table 19. Three of the organisms had original concentrations in the order of three to eight million spores per milliliter. T.A. 2285, however, had an extremely low count with the consequence that the results from this organism can only be considered as a trend.

From Table 19 it appears that the rate of irradiation, between 27,000 R/hr. and 367,000 R/hr. does not affect the total dosage in roentgens needed to decrease a spore population a given amount.

With a total irradiation below 300,000 R the two aerobes appear to be more resistant but as irradiation increases this difference was not found.

TABLE 19

Effect of Dosage Rate Upon Spore Survival

	Roentgens	<u>1518</u> 2,700,000/cc	<u>1503</u> 3,000,000/cc	<u>3814</u> 7,750,000/cc	<u>2285</u> 170/cc
	<u>213,000</u>	<u>27.04</u>	<u>30.00</u>	<u>4.53</u>	<u>5.8</u>
Cobalt 60					
187,000	961,000	0.0046	0.025	0.021	0
R/hr.	1,270,000	0.00022	0.003	0.0011	0
	1,500,000	0.000075	0.0008	0.00044	0
	1,760,000	0.000011	0.00001	0	0
	1,890,000	0.000011	0	0	0
	<u>137,000</u>	<u>49.26</u>	<u>83.33</u>	<u>18.06</u>	<u>9.3</u>
Tantalum					
182	411,000	1.30	4.10	4.74	0
27,000	822,000	0.0016	0.009	0.0027	0
R/hr.	1,230,000	0.0011	0.007	0.0015	0
	1,370,000	0	0.0001	0.0003	0
	1,710,000	0	0	0.00002	0
	<u>242,000</u>	<u>23.33</u>	<u>42.00</u>	<u>6.58</u>	<u>4.5</u>
Tantalum					
182	1,100,000	0.003	0.018	0.01	0
367,000	1,470,000	0	0.00027	0.0002	0
R/hr.	1,960,000	0	0.00001	0.0007	0
	2,200,000	0	0	0	0
	2,570,000	0	0	0	0

Discussion

There appears to be no difference between survival of spores in buffered phosphate and a 1:10 dilution of pea puree in water. This would be expected as the diluted pea puree in Experiment A had a specific gravity of 1.003. However, when 1:10 diluted pea puree and undiluted pea puree were compared, Experiment G, the undiluted puree offered some protection against irradiation over diluted puree. At this time the cause of this protection is open to conjecture. Some work in the literature (Burnett, 1951, 1952) has indicated a protective action of certain organic and inorganic chemicals. Lea (1947) reported a protective effect caused by proteinaceous material. These variables may be involved as well as available oxygen and others. The results imply that further work is needed to understand the protective mechanism involved.

The results of all the experiments indicate spore destruction by irradiation is approximately an exponential function of the amount of irradiation received (with extremely low or high dosages there was some trend toward a sigmoid curve). This is borne out in Experiment G where with varying original spore populations the percent survival is independent of the original population. This relationship of total roentgen dosage to percent survival is constant for a pure culture of a specific individual organism under specified conditions of irradiation. Each organism has a separate relationship as shown in Experiment A where the difference in resistance between T.A. 3814 and Cl.botulinum 62-A is marked. In order to determine more exactly the characteristics of this relationship for each of the test organisms, the data from several experiments were plotted in Graphs IV through VII. The difference of these survival curves

from pooled data as compared to individual experiments stresses the need for a large number of replicates before spore survival numbers can be reported or else more stringently controlled conditions of irradiation. Work is presently underway to better define these conditions needed for replication.

With these facts it is possible to estimate the amount of irradiation needed to reduce a spore population to an arbitrarily chosen commercial sterility level (i.e. 99.99%) regardless of the original population. However, to be able to predict the dosage needed to achieve total sterility of a spore population is another matter.

- (1) Burnett, W.T., Stapleton, G.E., Morse, M.L., and Hollaender, A., 1951. Reduction of sensitivity of Escherichia coli B/r by sulfhydryl compounds, alcohol, and sodium hydrosulfite. Proc. Soc. Exp. Biol. Med. 77, 636-638, 1951
- (2) Burnett, W.T., Morse, M.L., Burke, A.W., and Hollaender, A., 1952. Reduction of the X-ray sensitivity of Escherichia coli by sodium hydrosulfite and certain other inorganic sulphur compounds. J.BACT., 63 (5), 591-595, 1952
- (3) Lea, D.E., 1947. Action of Radiations on Living Cells, Macmillan Company, New York

Graph IV—Survival of *Cl. botulinum* 62A spores exposed to gamma radiation. (Composite).

Graph V—Survival of T.A. 3814 spores exposed to gamma radiation. (Composite).

Graph VI—Survival of P.A. 3679 spores exposed to gamma radiation. (Composite).

Graph VII—Survival of F.S. 1518 spores exposed to gamma radiation. (Composite)

Appendix and Glossary

The use of radiation as a sterilizing mechanism by the food technologist requires a general understanding of the forces involved and the types of radiations available. In the following brief review we have tried to bring out the major points relative to the understanding of the mechanism of sterilization by radiation.

A beam of radiation of any type is basically a source of energy in a highly available form. Exposure to such a beam for a given period of time allows the introduction into the irradiated volume of an amount of energy that, through computation, can be compared to other means of adding energy such as heat. The beams of radiation that might be of interest in sterilizing a microbiological population, using fission products, are (a) ionizing radiation, (b) electromagnetic radiation, and (c) neutron collisions. These all involve a collision of the type of radiation with some part of the atom in the material being irradiated.

The Atom

The atom is the smallest part of a chemical element which enters into a chemical reaction. It consists of two parts, (a) the nucleus and, (b) the outer ring or rings of electrons or electron shell.

The Proton

The proton is simply the nucleus of a light hydrogen atom with the electron in its electron shell stripped off. Protons are not usually found free in nature as there is such an abundance of electrons that a free proton would soon attract an electron and become a hydrogen atom. However, this can be accomplished in vacuo by stripping off the electron and accelerating the proton to produce a stream of "free protons" (as in the cyclotron).

The Electron

Electrons are the only particles which are found within the atom other than the nucleus. In a neutral atom the number of orbital electrons is exactly equal to the number of protons within the nucleus.

Ionization of an Atom

The process of removing an electron from an atom, resulting in a net charge of $1+$, is called ionization and the resulting atom is an ion. This can be accomplished, for instance, by shooting a high speed electron (Beta particle), from an outside source, at the atom. If the high speed electron were to hit, and knock out of the atom, an electron in the inner shell of electrons, creating a vacancy, an electron from the outer shell might jump inward and fill the vacancy. In this rearrangement of electrons within the atom, energy is liberated in the form of X-rays.

In general, the ability of a particle to cause ionization depends upon the nature of the particle (charge and mass), its velocity, and the state of medium which it transverses.

It is the ionization of matter that initiates the complex series of reactions that are able to produce death in microbiological populations. Hence, to cause primary damage to a microorganism any penetrating radiation must produce ionization. All charged particles, such as electrons and protons, readily produce ionization in tissue, but X-rays (and gamma rays) are sparsely ionizing and only a relatively few such rays which penetrate a microbial cell will produce ionization; the rest pass harmlessly through the cell.

I Ionizing Radiations

These radiations consist of the alpha and beta particles, both of which act directly (ionize) by applying their kinetic energy or electrical force on the neutral atom and dislodge an orbital electron.

(a) Alpha Particles

Alpha particles are the nucleus of helium atoms obtained by spontaneous emission from radioactive material or artificially with the cyclotron. Their range in the penetration of liquids or solids is extremely limited and hence their usefulness is limited to material exposed in thin films. When emitted from nuclei they are of the order of 5 M.E.V. of energy and are classed as high velocity particles. They are composed of 2 neutrons and 2 protons and compared to electrons. Such nuclei are massive resulting in easy absorption in tissue and low penetration power.

(b) Beta Particles

Beta particles are high velocity electrons emitted by radioactive decaying materials or artificially accelerated as cathode rays. When the Beta particles are emitted from radioactive atoms their velocity is of the order of 1 M.E.V. of energy. Similar to Alpha particles, Beta particles, although of high velocity, have limited penetration power. A few sheets of paper will stop alpha particles while beta particles are completely absorbed in several millimeters of water or aluminum.

II Electromagnetic Radiations

These radiations consist (in terms of fission product use) of X-rays and gamma rays. They act indirectly (ionize) by the transmission of a quantum of energy to the atom, the result of which is the acceleration of a secondary charged particle (an electron from the bombarded atom) producing ionization. This process, the "photo electric effect", is one of

three methods (Compton Effect and Pair Production) by which photons produce ionization.

(a) X-rays

X-rays are an electromagnetic radiation emitted in quanta (as in the much longer wave ultra violet light) and the packet of this energy is expressed as a photon. X-rays are produced by the change of an electron from an outer to an inner ring of an atom. Because the electrons in various atoms are bound to their respective nuclei with different energies, the energy characteristic of any given photon is governed by the parent element from which it arose.

Man-made X-rays are produced by allowing a stream of high energy electrons to impinge upon a target such as tungston. This bombardment produces an electron rearrangement within the tungston atom which results in the emission of X-rays.

X-rays themselves are non-ionizing, but when they stride an atom, the energy of the photon is transferred (in one of three ways) to an electron within the atom which then becomes a high energy free electron, and produces ionization.

(b) Gamma Rays

Gamma rays are naturally occurring X-rays, however, they originate from a different site within the atom. These two rays appear to have the same properties in regard to biological effect.

X-rays produced by electrons are accelerated at less than 0.1 M.E.V., are termed soft X-rays, and have less penetrating power than hard X-rays (more than 0.15 M.E.V.). "Super voltage" X-rays are produced at more than 1.0 M.E.V.

Thus we see that the damage or effect of radiations of all types, on biological material, depends on the absorption of the energy of the radiation by the biological material, or in other words, by ionization. This is shown by the alpha particles being about 100 times as effective in injuring biological systems as beta particles. Both are less penetrating and more destructive than gamma rays or hard X-rays. This is in close agreement with their ionizing powers (alpha 10,000, beta 1000, gamma 1) and inversely with their range.

Units of Measurement

The roentgen (R) is a unit of measure for X-rays and basically is a measure of the number of ionizations per gram of air. Since this is a unit of absorption of X-rays it would be difficult to express ionization by other types of radiation in terms of roentgens due to the difference in wave length or absorption coefficient. Hence, the REP (roentgen equivalent physical) has developed to express the ionization taking place in equivalent terms. This is the amount of radiation which produces energy absorption of 93 ergs per gm. of tissue.

THIS PAGE
WAS INTENTIONALLY
LEFT BLANK