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5650 South Ellis Avenue
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REPORT

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5650 S. Ellis Avenue
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Principal Investigators:

R.O. Wagenaar
H. Sugiyama
M.J. Surgalla

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- (a) Study on the effect of radiation on heat resistance of bacterial spores.
- (b) Study of the effect of radiation on staphylococcus enterotoxin.
- (c) Radiation studies on cheese spreads.

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(a)

Study on the Effect of Radiation on Heat Resistance of Bacterial
Spores

Personnel: H. Sugiyama, Research Associate (Bacteriologist)

Objectives: To determine the relationship between the conditions of sporulation and resistance to radiation, the relationship between heat resistance of spores and resistance to radiation, and the effect of radiation on heat resistance of spores.

Summary:

EFFECT OF RADIATION ON HEAT RESISTANCE OF CLOSTRIDIUM
BOTULINUM SPORES

It was originally planned that the study of radiation effects on spores would coincide with a separate study on heat resistance. In this way the data on heat resistance obtained at no expense to the E.E.C. project would be used as control data for studies on effects of radiation on heat resistance. This work has been delayed due to technical difficulties in making the desired improvements on a medium to be used in the work.

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(b)

Study of the Effect of Radiation on Staphylococcus Enterotoxin

Personnel: M.J. Surgalla, Research Associate (Bacteriologist)

Objectives: To determine radiation levels necessary to inactivate enterotoxin, and to determine the effect of sublethal radiation doses on enterotoxigenicity of staphylococcal cultures.

Summary:

Partially purified enterotoxin has been irradiated at three different dosage levels and assayed by the monkey feeding test. Enterotoxin was inactivated at a dosage of 1,800,000 rep. but not by 160,000 or 460,000 rep. See Table 1.

Table 1

Inactivation of Staphylococcal Enterotoxin by Irradiation with Cobalt 60

Experiment Number	Irradiation				Toxin Assay in Macaca mulatta			
	Mg.	ml.	Hrs.	Rep.	Sample	Dose in terms of Crude toxin fed.	No pos. Used Animals	No. Fed New Animals
1	550	27.5	18.5	1,800,000	Irradiated Control Control	40 ml 40 ml 8 ml	0/18 3/6 0/5	
2	550	30	1.75	160,000	Irradiated Control	40 ml 40 ml	1/9 1/10	2/3 1/2
3	550	30	5.0	460,000	Irradiated Control	40 ml 40 ml	2/3 0/3	1/3 2/3

The enterotoxin was produced in deep aerated culture in amigen medium, precipitated at pH 3.5 with phosphoric acid, dialyzed against distilled water, and lyophilized (yield 55 mg/100 ml). The toxin was dissolved in saline buffered

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with 0.02 M phosphate (pH 7.3). Samples were irradiated at 3 - 8° C. in 5x50 cm pyrex glass tubes with cork stopper, and were assayed by the monkey feeding test on the next day along with irradiated controls. The remaining materials were lyophilized and fed again if necessary.

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(c)

Radiation Studies on Cheese Spreads

Personnel: R.O. Wagenaar, Research Associate (Bacteriologist)

Objectives: To determine the feasibility of using radiation in safeguarding vacuum packed cheese spreads.

Summary:

Data from 14 experiments on soft surface ripened cheese samples inoculated with Clostridium botulinum spores and irradiated at various dosages of gamma irradiation with Cobalt-60 are presented. There were some variations in the levels of irradiation tolerated by spores of the same strain of Cl. botulinum when tested in separate experiments. The data showed that spores of culture 62A were somewhat more resistant to irradiation than the spores of either of the other type A strains tested. The spores of one type B strain were inactivated by lower dosages of irradiation than were type A spores. In general, type A spores were inactivated by 800,000 to 1,100,000 rep.; type B spores by 550,000 rep. Results from experiments designed to study the effect of irradiation with Cobalt-60 on the toxin-producing ability of spores showed that this characteristic was unaltered by sub-lethal dosages.

Preliminary experiments designed to test the effect of gamma irradiation on Cl. botulinum toxin inoculated into surface-ripened cheese showed that levels of irradiation much higher than those anticipated were required to inactivate botulinum toxin in this medium. Similar results were observed when crude toxin preparations grown in either trypticase or beef heart broths were irradiated with Cobalt-60. In the trials with broth or cheese containing Cl. botulinum toxin, the toxicity was assayed at three levels; namely, 1,000, 200 and 20 m.l.d. Insufficient samples of some of the experimental series were available to reach the proposed endpoint (less than 20 m.l.d.); hence, specific dosages required for inactivation were not established in all trials. There was no apparent differential influence on the rate of inactivation of the toxin

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in trypticase broth whether the atmosphere above the broth in the sealed tubes was CO₂, a mixture of 90% N₂ and 10% CO₂, or air. Similarly, the pattern of inactivation for toxin in beef heart broth appeared to be the same as for the toxin in trypticase broth. In broth, where the initial toxin level was somewhat above 20 m.l.d., reduction of the toxicity to the endpoint (less than 20 m.l.d.) required at least 2,200,000 rep. When the initial toxicity was somewhat above 200 m.l.d. reduction to below 20 m.l.d. required at least 4,200,000 rep. Toxin levels above 1,000 m.l.d. were not reduced to below 20 m.l.d. by less than 4,300,000 rep., and in most instances at least 5,300,000 rep. were required.

RADIATION STUDIES ON SURFACE RIPENED CHEESE

At the time of the last report (No. 2, covering the period 18 June 1952 to 30 June 1953), some of the experiments on Clostridium botulinum spores irradiated in surface ripened cheese were still in progress. Data on additional experiments of this type will be presented in this report. Most of our more recent studies have been on the effect of gamma irradiation with Cobalt-60 on Cl. botulinum toxin in both broth and surface ripened cheese mediums. The extremely high dosages of irradiation required to inactivate these toxic preparations was somewhat unexpected. These experiments were designed to determine: (1) the irradiation dosage required to reduce the toxicity of cheese or broth samples containing varying initial toxin levels to the minimum level measurable by intraperitoneal mouse injections (in this instance to less than 20 m.l.d. per gram of sample), and (2) the general pattern of reduction in toxicity as progressively higher doses of irradiation were applied to similar toxic samples.

Materials and Methods

In the experiments in which spores were to be irradiated, moderately ripened Liederkrantz cheese diluted with approximately 10% distilled water was warmed in a water bath at 70° C. and stirred until homogeneous. A sufficient quantity of well-mixed cheese was accurately weighed into a beaker; then, the cheese was pasteurized in a water bath at 90° C. for 10 minutes. Immediately after pasteurization the cheese was cooled to below 60° C. Sterile distilled water was then added to restore the moisture lost during pasteurization. After inoculating the cheese with 10,000 per gram of toxin-free spores, 6 gram portions were weighed into sterile 15 x 125 mm. culture tubes. The section of the tube approximately one inch above the top level of the cheese was constricted; then, the air from above the cheese was evacuated and replaced with a mixture of 90% N₂ and 10% CO₂. The final vacuum level in the sealed tubes was 400 mm. Hg.

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Six sealed tubes were irradiated at each exposure. Analyses for Cl. botulinum toxin were conducted on samples inoculated with type A spores after 3, 7, 14, 30 and 60 days of incubation at 30° C.; with type B the analyses were at 7, 14, 30, 60 and 90 days. At each analysis an unirradiated control sample was tested in parallel with the irradiated specimen. In addition, an uninoculated unirradiated sample was tested with each series. Toxin was assayed by intraperitoneal injection of white mice with 0.3 ml. portions of the supernatant fluid from centrifuged 1 to 5 aqueous dilutions of the cheese.

In the experiments in which broth containing Cl. botulinum toxin was added to cheese, Liederkranz cheese was warmed in a water bath at 70° C. and stirred until homogeneous. The cheese was weighed into a beaker, pasteurized and cooled to below 50° C. Sterile distilled water was added to restore the moisture lost during pasteurization. Sufficient Seitz-filtered toxic broth (usually 10 or 15%) was added to yield the desired toxin level in the cheese. Four gram portions were weighed into sterile 15 x 125 mm. culture tubes. The air above the cheese was partially evacuated. In some experiments, the air was replaced with a mixture of 90% N₂ and 10% CO₂, and in other cases with CO₂. The vacuum in the tubes was 400 mm. Hg before sealing.

The air above the toxic broth was evacuated to 400 mm. Hg. In two experiments, the air was replaced with a mixture of 90% N₂ and 10% CO₂.

The toxin in the cheese and broth samples was assayed at three levels; namely, 1000, 200 and 20 m.l.d. The lowest level that could be detected in cheese was 20 m.l.d. without possibly encountering non-specific deaths from protein breakdown products. To keep the data comparable, similar titration levels were used for the broth samples.

TABLE 2

Toxin Production in Liederkrantz Cheese Experimentally Inoculated with Toxin-Free Spores of Type A Clostridium botulinum and after Irradiation with Cobalt-60^a
(Inoculum -- 10,000 spores per gram)

Expt.	Culture Used	Hours Exposure	Dosage Rep.	Days of Incubation at 30° C.				
				3	7	14	30	60
G	T	8.2	820,000	L*	L	L	L	L
G	T	5.5	550,000	L	L	L	L	L
G	T	4.1	410,000	L	D*	D	D	D
K	T	11.0	1,100,000	L	L	L	L	L
K	T	8.2	810,000	L	D	D	D	D
K	T	5.5	540,000	L	D	D	D	D
K	T	4.1	410,000	D	D	D	D	D
O	T	11.0	1,100,000	L	L	L	L	L
O	T	8.2	800,000	L	L	L	L	L
O	T	5.5	540,000	L	D	D	D	D
O	T	4.1	400,000	D	D	D	D	D
E	62A	16.5	1,700,000	L	L	L	L	L
E	62A	8.2	830,000	L	D	D	D	D
E	62A	5.5	550,000	L	D	D	D	D
E	62A	4.1	410,000	L	D	D	D	D
H	62A	11.0	1,100,000	L	L	L	L	L
H	62A	8.2	820,000	L	D	D	D	D
H	62A	5.5	550,000	D	D	D	D	D
L	62A	13.6	1,300,000	L	L	L	L	L
L	62A	11.0	1,100,000	L	L	L	L	L
L	62A	8.2	810,000	L	D	D	D	D
L	62A	5.5	540,000	D	D	D	D	D
P	62A	13.6	1,300,000	L	L	L	L	L
P	62A	11.0	1,100,000	L	L	L	L	L
P	62A	8.2	800,000	L	D	D	D	D
P	62A	5.5	540,000	D	D	D	D	D
F	109A	16.5	1,700,000	L	L	L	L	L
F	109A	8.2	830,000	L	L	L	L	L
F	109A	4.1	410,000	L	L	D	D	D
I	109A	11.0	1,100,000	L	L	L	L	L
I	109A	8.2	820,000	L	L	D	D	L
I	109A	5.5	550,000	D	D	D	D	D
I	109A	4.1	410,000	D	D	D	D	D
M	109A	11.0	1,100,000	L	L	L	L	L
M	109A	8.2	800,000	L	L	D	L	L
M	109A	5.5	540,000	L	D	D	D	D
M	109A	4.1	400,000	D	D	D	D	D

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TABLE 2 (cont.)

Expt.	Culture Used	Hours Exposure	Dosage Rep.	Days of Incubation at 30° C.				
				3	7	14	30	60
Q	109A	11.0	1,100,000	L	L	L	L	L
Q	109A	8.2	790,000	L	L	L	L	L
Q	109A	5.5	530,000	D	D	D	D	D
Q	109A	4.1	400,000	D	D	D	D	D

L = Lived

D = Died

^a The temperature of irradiation varied between 3.5° and 8.0° C.

TABLE 3

Toxin Production in Liederkrantz Cheese Experimentally Inoculated with Toxin-Free Spores of Type B Clostridium botulinum and after Irradiation with Cobalt-60^a
(Inoculum -- 10,000 spores per gram)

Expt.	Culture Used	Hours Exposure	Dosage Rep.	Days of Incubation at 30° C.				
				7	14	30	60	90
D	126.7B	16.5	1,700,000	L*	L	L	L	L
D	126.7B	8.2	830,000	L	L	L	L	L
D	126.7B	4.1	410,000	L	D*	L	L	D
J	126.7B	11.0	1,100,000	L	L	L	L	L
J	126.7B	8.2	810,000	L	L	L	L	L
J	126.7B	5.5	540,000	L	L	L	L	L
J	126.7B	4.4	440,000	L	L	L	L	L
N	126.7B	8.2	800,000	L	L	L	L	L
N	126.7B	5.5	540,000	L	L	L	L	L
N	126.7B	4.1	400,000	L	L	L	D	D
N	126.7B	2.85	280,000	L	L	D	D	D

L = Lived

D = Died

^a The temperature of irradiation varied between 3.5° and 8.0° C.

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Effect of Irradiating Spores of Clostridium botulinum in Surface Ripened Cheese

Data from 14 experiments on soft surface ripened cheese samples inoculated with Cl. botulinum spores and irradiated at various dosages of gamma irradiation with Cobalt-60 are shown in Tables 2 and 3. The irradiation levels that inhibited growth and toxin production by each of 3 Type A strains are shown in Table 2, and similar information on 1 type B strain is presented in Table 3. Calibration data furnished by the Health Physics Department of the University of Chicago was used in calculating the irradiation dosages.

The data in Table 2 showed that there were some variations in the levels of gamma irradiation tolerated by spores of the same strain of Cl. botulinum when tested in separate experiments. Three trials with culture T showed that in Experiment G the spores survived 410,000 rep., but were inactivated by 550,000 rep. In Experiment K they survived 810,000 rep., but were inactivated by 1,100,000 rep. Results from Experiment O showed a tolerance between the values obtained in the two previous trials; namely, that the spores of strain T survived 540,000 rep., but were inactivated by 800,000 rep. The results of the four trials with culture 62A showed good agreement. In Experiments E, H, L and P the spores survived approximately 800,000 rep., but were inactivated by 1,100,000 rep. Variability similar to that observed with culture T was shown by culture 109A in four comparable trials. In Experiment F spores of culture 109A survived 410,000 rep., but were inactivated by 830,000 rep. In Experiment I they survived 820,000 rep., but not 1,100,000 rep. In the latter experiment, a sample exposed to 820,000 rep. was negative for toxin at the 60-day analysis after samples from the same series were toxic at the 14- and 30-day analyses. It is possible that a small amount of toxin was present here, also, but at a level too low to be detected in 0.3 ml. of a 1 to 5 dilution of the test sample. In Experiment M spores of culture 109A survived 540,000 rep., but not 800,000 rep. (the 14-day sample exposed to the higher level of irradiation contained toxin, but all others in the series were negative). The spores in Experiment Q survived 530,000 rep.,

but not 790,000 rep.

The data in Table 3 showed that the spores of the one type B strain of Cl. botulinum tested (126.7B) were less resistant than type A spores when irradiated in cheese. In Experiment D toxin was demonstrated at two analysis intervals (14 and 90 days) in cheese exposed to 410,000 rep. All other analyses on samples in this series showed insufficient toxin in 0.3 ml. of a 1 to 5 dilution to kill mice. In Experiment J all samples exposed to 440,000 rep. were negative for toxin. Data from Experiment N showed that spores of culture 126.7B survived 400,000 rep., but were inactivated by 540,000 rep. Toxic samples were demonstrated only after 60 and 90 days of incubation at 30° C.

The results on the three type A strains of Cl. botulinum tested showed that incubation at 30° C. beyond 7 days only rarely increased the irradiation level at which toxin was produced, and that had occurred only with spores of culture 109A. This observation indicated that irradiated type A spores not capable of initiating growth in the cheese within the first week of incubation probably will not grow and produce toxin. No such clean cut indications were given by the type B spores tested.

These experiments showed that toxin production by Cl. botulinum spores was inhibited in Liederkranz cheese at lower levels of gamma irradiation than had been previously reported for similar spores irradiated in other mediums. There is a possibility that products toxic to Cl. botulinum were formed in the cheese which inhibited growth and toxin production of spores still surviving a particular level of irradiation. From the safety standpoint an irradiation level above the highest one which permitted growth must be used in any recommendation of a sterilization irradiation dosage for cheese.

In an attempt to estimate what percentage of the inoculated spores survived varying levels of irradiation, spore counts from cheese tubes exposed to irradiation dosages near the highest level which permitted toxin production were made. These data are shown in Table 4.

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TABLE 4

Type A Clostridium botulinum Spores in Liederkrantz Cheese Surviving
Varying Levels of Irradiation with Cobalt-60
(Initial Inoculum - 10,000 spores per gram)

Expt.	Culture Used	Hours Exposure	Dosage Rep.	Survivors Spores per gram	Percent Survival	Toxin Produced in Cheese
K	T	5.5	540,000	1,500	15.0	Yes
K	T	4.1	410,000	4,400	44.0	Yes
O	T	5.5	540,000	1,900	19.0	Yes
O	T	4.1	400,000	Counts Unsatisfactory		Yes
L	62A	11.0	1,100,000	< 10	< 0.1	No
L	62A	8.2	810,000	140	1.4	Yes
P	62A	11.0	1,100,000	< 10	< 0.1	No
P	62A	8.2	800,000	300	3.0	Yes
M	109A	5.5	540,000	550	5.5	Yes
M	109A	4.1	400,000	Counts Unsatisfactory		Yes
Q	109A	5.5	530,000	700	7.0	Yes
Q	109A	4.1	400,000	Counts Unsatisfactory		Yes

These data showed that spore counts varying from 140 to 1,900 per gram were still present in cheese samples exposed to irradiation dosages near the highest level which permitted consistent toxin production in the respective experiments for which the information was recorded. In Experiment K toxin was produced in samples irradiated at one level higher (810,000 rep.) than was checked by spore recovery counts. Also, in Experiment M one toxic sample (at 14 days) was demonstrated after irradiation with 800,000 rep. There was good agreement in the survival counts between each pair of samples irradiated at similar dosage levels. The toxin production data in Table 2, together with the data in Table 4, showed that culture 62A was more resistant to irradiation with Cobalt-60 than either of the other two type A cultures tested.

Results from experiments designed to study the effect of irradiation on the toxin producing ability of spores showed that this characteristic was unaltered by sub-lethal dosages. Spores that survived a dosage of irradiation slightly below that which inhibited growth and toxin production when the irradiated cheese

was incubated at 30° C. yielded colonies in agar that produced Cl. botulinum toxin in beef heart broth of a potency equal to the titre of similar toxin from non-irradiated Cl. botulinum spores.

Effect of Irradiating Toxin of Clostridium botulinum in Surface Ripened Cheese

Preliminary experiments designed to test the effect of gamma irradiation on Cl. botulinum toxin inoculated into surface ripened cheese showed that levels of irradiation much higher than those anticipated were required to inactivate botulinum toxin in this medium. In the initial experiments, cheese containing toxin that had been produced in trypticase broth and which had a toxicity of approximately 1,000 m.l.d. was not reduced to a toxicity of 200 m.l.d. (the next lowest test level) by irradiation with 770,000 rep. This was observed with toxin from both cultures T and 109A (both type A). Two experiments were conducted with toxin from culture 126.7B (type B). In neither instance did irradiation with 770,000 rep. reduce the toxicity from 1,000 to 200 m.l.d.

Effect of Irradiating Toxin of Clostridium botulinum in Trypticase Broth

After observing the slow inactivation of Cl. botulinum toxin in cheese by irradiation with Cobalt-60, it was suspected that this medium possibly contributed some protection to the toxin. Therefore, several experiments were conducted in which Cl. botulinum toxin in trypticase broth was irradiated at varying dosages.

The data on some of the earlier trials with trypticase broth containing Cl. botulinum toxin did not extend to irradiation dosages high enough to include the reduction of the toxicity of the test samples to below 20 m.l.d. This level was chosen as the lowest test point so that these results would be comparable to similar data on Cl. botulinum toxin added to cheese. In the interpretation of the data presented in Tables 5,6,7 and 8 it should be pointed out that toxin was assayed for by duplicate intraperitoneal injections into mice at each of three levels; namely, 1,000, 200 and 20 m.l.d. Therefore, in the tables, 1,000 m.l.d. indicates 1,000 m.l.d. or more (but below 10,000 m.l.d.), 200 m.l.d. indicates

between 200 and 1,000 m.l.d. and 20 m.l.d indicates between 20 and 200 m.l.d. In Tables 5 and 6 data are presented from eight experiments with toxic trypticase broths having a low initial toxicity. For those experiments shown in Table 5, the toxin was produced by culture T; the data in Table 6 are from toxic broths produced by culture 109A. In these trials the atmosphere above the broth in the partially filled sealed tubes was varied to test its effect on the susceptibility of the crude toxin to gamma irradiation.

TABLE 5

The Effect of Varying the Atmosphere Above Toxic Trypticase Broth in Partially Filled Sealed Tubes on the Inactivation of Clostridium botulinum Toxin Irradiated with Cobalt-60

(Toxin produced by culture T - type A)

Irradiation Dosage Rep.	Atmosphere Above Broth in Tube			
	CO ₂	90% N ₂ +10% CO ₂	Air Trial	Air Trial
	m.l.d. Toxin	m.l.d. Toxin	1 m.l.d. Toxin	2 m.l.d. Toxin
None	20 ^a	20	20	20
370,000	20	20	20	20
560,000	20	20	20	20
740,000	20	20	20	20
1,100,000	20	20	20	20
1,500,000	20	20	20	20
1,800,000		20	20	20
2,200,000		20	20	<20
3,300,000				<20
3,800,000				<20
4,400,000		<20 ^b		

a This means between 20 and 200 m.l.d.

b This means less than 20 m.l.d.

TABLE 6

The Effect of Varying the Atmosphere Above Toxic Trypticase Broth in Partially Filled Sealed Tubes on the Inactivation of Clostridium botulinum Toxin Irradiated with Cobalt-60

(Toxin produced by culture 109A - type A)

Irradiation Dosage Rep.	CO ₂ m.l.d. Toxin	Atmosphere Above Broth in Tube		Air Trial 1 m.l.d. Toxin	Air Trial 2 m.l.d. Toxin
		90% N ₂ +10% CO ₂ m.l.d. Toxin			
None	20 ^a	20		200 ^c	20
370,000	20	20		200	20
560,000	20	20		200	20
740,000	20	20		20	20
1,100,000	20	20		220	20
1,500,000	20	20		220	20
1,800,000		20		220	20
2,200,000		20		220	20
3,300,000					20
3,800,000					20
4,400,000		<20 ^b			

a This means between 20 and 200 m.l.d.

b This means less than 20 m.l.d.

c This means between 200 and 1,000 m.l.d.

An examination of the data in Tables 5 and 6 showed that there was no apparent differential influence of any of these atmospheres on the rate of inactivation of the toxin in the broth. These data further showed that with broth containing between 20 and 200 m.l.d. of Cl. botulinum toxin at least 2,200,000 rep. were required to reduce the toxicity to below 20 m.l.d. In another experiment, in which the initial toxicity of the broth was probably very near 200 m.l.d., the toxin was not reduced to below 20 m.l.d. by a dosage of 3,800,000 rep. Insufficient samples of some of the experimental series were available to reach the proposed endpoint; hence, specific dosages required for inactivation were not established in all trials. The large dosages of gamma irradiation required to inactivate Cl. botulinum toxin was in itself somewhat unexpected, because of the heat lability of this toxin.

TABLE 7

The Effect of Varying Dosages of Irradiation with Cobalt-60 on Clostridium botulinum Toxin in Trypticase Broth in Partially Filled Sealed Tubes

(Atmosphere over broth in tubes was air)

Irradiation Dosage Rep.	Toxin Produced By	
	Culture 62A (Type A) m.l.d. Toxin	Culture 126.7B (Type B) m.l.d. Toxin
None	200 ^a	200
360,000	20 ^b	200
550,000	20	200
730,000	20	200
1,100,000	20	200
1,500,000	20	200
1,800,000	20	200
2,200,000	20	200
3,300,000	20	20

^a This means between 200 and 1,000 m.l.d.

^b This means between 20 and 200 m.l.d.

Data on a single experiment with each of cultures 62A (type A) and 126.7B (type B) are presented in Table 7. The initial toxin levels were somewhere between 200 and 1,000 m.l.d. These results showed an inactivation pattern similar to that observed with toxic broths from the other two type A cultures, although in these experiments the initial toxin levels were higher than was the case with most of the trials shown in Tables 5 and 6. Because of the broad ranges of toxicity between m.l.d. titration levels, the data are not detailed enough to predict accurately whether toxin from any one of the Cl. botulinum strains tested was more resistant to irradiation than the others.

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TABLE 8

The Effect of Varying Dosages of Irradiation with Cobalt-60 on Clostridium botulinum Toxin in Trypticase Broth at a Lowered pH Level and in Partially Filled Sealed Tubes^a

(Atmosphere over broth in tubes was air)

Irradiation Dosage Rep.	Toxin Produced By	
	Culture T (type A) m.l.d. Toxin	Culture 109A (type A) m.l.d. Toxin
None	200 ^b	1,000 ^e
350,000	200	1,000
530,000	200	1,000
700,000	200	200
1,100,000	200	200
1,400,000	200	200
1,800,000	20 ^c	200
2,100,000	20	200
3,200,000	20	200
4,200,000	<20 ^d	20
5,300,000	<20	<20

^a The pH of the broth in all tubes was approximately 5.50.

^b This means between 200 and 1,000 m.l.d.

^c This means between 20 and 200 m.l.d.

^d This means less than 20 m.l.d.

^e This means between 1,000 and 10,000 m.l.d.

In Table 8 data are shown for two experiments in which the pH levels of toxic trypticase broths were adjusted downward from approximately 7.50 to near pH 5.50 with HCl. The general pattern of inactivation of the toxin appeared to be very similar to that for broths at pH levels nearer neutrality, except that the rate of inactivation seemed to increase somewhat at the higher irradiation dosage levels. Additional trials would be needed to establish this observation conclusively. The dosage required to reduce the toxicity to below 20 m.l.d. was 4,200,000 rep. when the initial level was between 200 and 1,000 m.l.d. and 5,300,000 rep. when the initial level was above 1,000 m.l.d.

In an attempt to obtain some broth cultures with a higher initial toxicity, cultures T and 109A were grown in beef heart broth with meat present. Data from four experiments with toxic beef heart broths are presented in Table 9.

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TABLE 9

The Effect of Varying Dosages of Irradiation with Cobalt-60 on Clostridium botulinum Toxin in Beef Heart Broth in Partially Filled Sealed Tubes

(Atmosphere over broth in tubes was air)

Irradiation Dosage Rep.	Toxin Produced By			
	Culture T	Culture 109A (type A)		
	Trial 1 m.l.d. Toxin	Trial 2 m.l.d. Toxin	Trial 1 m.l.d. Toxin	Trial 2 m.l.d. Toxin
None	1,000 ^a	1,000	1,000	1,000
360,000	1,000	200	1,000	1,000
540,000	200 ^b	200	1,000	1,000
720,000	200	200	1,000	1,000
1,100,000	200	200	200	1,000
1,400,000	200	200	200	1,000
1,800,000	200	200	200	1,000
2,200,000	200	20	200	1,000
2,700,000	20 ^c		200	
3,200,000	20	20	20	200
3,800,000	20		20	
4,300,000	20	<20 ^d	20	20
5,300,000		<20		20

a This means between 1,000 and 10,000 m.l.d.

b This means between 200 and 1,000 m.l.d.

c This means between 20 and 200 m.l.d.

d This means less than 20 m.l.d.

The data in Table 9 showed that the general pattern of inactivation of the toxin was the same in these experiments as was observed with the toxic trypticase broths. The higher initial toxicity of the beef heart broth samples irradiated in these trials yielded higher irradiation levels for the reduction of the toxin to 20 m.l.d. (or less than 20 m.l.d. in one trial). In Trial 1 with toxic broth produced by culture T, 4,300,000 rep. was insufficient to reduce the toxicity from somewhat above 1,000 m.l.d. to below 20 m.l.d. In the second trial a toxic broth from culture T, which apparently had an initial toxicity very near 1,000 m.l.d., was reduced to below 20 m.l.d. by 4,300,000 rep. The toxic broths produced by culture 109A had higher initial toxin levels than those from culture T. In trial 1 with toxic broth from this source, the toxin level was not reduced from above 1,000 m.l.d. to below 20 m.l.d. by 4,300,000 rep. The second broth

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sample from culture 109A had an even higher initial toxin level that was not reduced to below 20 m.l.d. by 5,300,000 rep.

The data from the several experiments with broths containing Cl. botulinum toxin showed that there was an orderly destruction of the toxicity during the extended irradiation exposure required to reduce the varying initial amounts of toxin to the proposed endpoint (less than 20 m.l.d.). In the experiments where the initial toxin level was somewhat above 20 m.l.d., reduction of the toxicity to the endpoint required at least 2,200,000 rep. When the initial toxicity was somewhat above 200 m.l.d. reduction to below 20 m.l.d. required at least 4,200,000 rep. Toxin levels above 1,000 m.l.d. were not reduced to below 20 m.l.d. by less than 4,300,000 rep., and in most instances at least 5,300,000 rep. were required.

The Effect upon the Titre of Botulinum Toxin Mixed in Irradiated Cheese and Broth and Stored at 3° C.

There was a possibility that some of the effects of irradiation with Cobalt-60 would be transient and reversible. As a check to see if samples irradiated with varying dosages of gamma irradiation maintained their initial toxicity, a duplicate set of samples from each experiment was analyzed 2 weeks after the initial analyses were conducted. In a few instances there was an apparent increase in toxicity, although more often there was an apparent decrease in the toxin level. However, approximately eighty per cent of the samples had the same toxin level initially and after 2 weeks of storage at 3° C.

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