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Progress Report

DETERMINATION OF RADIATION STERILIZATION DOSE FOR CANNED MEAT

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SUMMARY

All phases of the project are now under investigation. Conclusions, however, are premature at present because sufficient incubation time has not yet elapsed to establish even tentative results.

THIS IS NOT A FINAL REPORT. CONCLUSIONS STATED ARE SUBJECT TO CHANGE ON THE BASIS OF ADDITIONAL EVIDENCE. THIS INFORMATION IS NOT TO BE PUBLISHED WITHOUT WRITTEN PERMISSION FROM HQ, QM R AND D COMMAND, NATICK, MASS.

PHASE I

EFFECT OF PREIRRADIATION OF CANNED GREEN PEAS ON THE F_0 SUBSEQUENTLY REQUIRED FOR STERILIZATION

Combined irradiation-heat processing may conceivably be applied to other foods than meat. The benefits to be logically expected would follow from possible improvement of organoleptic qualities. A suitable test food for this purpose should be one that stimulates germination and subsequent growth of putrefactive anaerobic spores that are significant in food spoilage. Green peas are often used as the base of germination and culture media needed for such spores and in addition, canned green peas are a staple item of human consumption. Hence green peas were selected for study.

The combined irradiation-heat processing treatments required to sterilize canned green peas inoculated with 300PA3679 spores per can were presented in Progress Report No. 1 and are summarized here for reference in Fig. 1.

This study has been continued with a second series of runs using 5,000,000 C. botulinum 213B spores per can. The canning, irradiation, and heat processing treatments used were described in Progress Report No. 1.

RESULTS

Data from runs PB-1 through PB-17 are detailed in the table and plotted in the figure. Conclusions are not drawn since longer incubation is required before they would be meaningful.

TABLE

F₀ VALUES REQUIRED TO STERILIZE CANNED GREEN PEAS PACKED IN NO. 1 PICNIC TIN CANS, INOCULATED WITH 5,000,000 *C. botulinum* 213B SPORES PER CAN, AND IRRADIATED WITH GAMMA RAYS FROM COBALT-60 BEFORE HEAT PROCESSING AT 230°F AND INCUBATION AT 85°F

Run No.: PB-1
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 *C. botulinum* 213B Spores Per Can
 Irradiation: None
 Processing Temperature: 230°F
 Incubation: 85°F

F ₀	Can No.	Days-to-gas Formation	*Toxin
Inoculated controls	1	2	
	2	2	
Noninoculated controls	1	3	
	2	3	
Can 1, 1.03	5	9	2/2
Can 2, 1.03	6	11	2/2
Can 3, 1.03	7	10	2/2
	8	12	2/2
Can 1, 0.63	9	10	
Can 2, 0.63	10	10	
Can 3, 0.63	11	10	
	12	9	
Can 1, 0.52	13	8	
Can 2, 0.52	14	9	
	15	9	
	16	9	
Can 1, 0.29	17	5	
Can 2, 0.29	18	9	
	19	6	
	20	9	
Can 1, 0.26	21	5	2/2
Can 2, 0.26	22	5	2/2
	23	6	2/2
	24	6	2/2
Can 1, 1.44	25	24	
	26	13	2/2
	27	24	
	28	13	2/2

*Toxin determined by intraperitoneal injection of 0.5 ml of juice into mice (2/2 means 2 dead mice out of 2 injected).

Conclusion: An F₀ in excess of 1.44 is required to sterilize the canned peas.

TABLE (Continued)

Run No.: PB-2
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: As Indicated
 Processing Temperature: None
 Incubation Temperature: 85°F

Megarad	Can No.	Days-to-gas Formation	Toxin
1.530	13	6	
	14	9	
	15	9	
	16	9	
2.045	9	-	
	10	8	
	11	16	0/2
	12	-	
3.080	1	-	
	2	-	
	3	-	
	4	-	
3.600	5	-	
	6	-	
	7	17	2/2
	8	17	2/2

Conclusion: Provided that no error was made in recording the radiation dosage for cans 1 through 8, it appears that more than 3.60 megarad are needed for sterilization. It seems more likely that the dosage is between 3.08 and 3.60 megarad, however.

Note: Same controls as used for Run PB-1.

TABLE (Continued)

Run No.: PB-3
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: 0.465 megarad
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation	Toxin
Can 1, 1.53	1	-	
Can 2, 1.53	2	-	
Can 3, 1.53	3	-	
	4	-	
	5	11	0/2
Can 1, 1.03	6	11	0/2
Can 2, 1.03	7	15	2/2
Can 3, 1.03	8	15	2/2
Can 1, 0.70	9	16	
Can 2, 0.70	10	20	
Can 3, 0.70	11	13	2/2
	12	26	
Can 1, 0.46	13	9	
Can 2, 0.46	14	17	
	15	13	
	16	9	
Can 1, 0.26	17	9	
Can 2, 0.26	18	13	
	19	13	
	20	9	

Conclusion: Under these conditions, canned green peas were sterilized by 0.465 megarad of gamma radiation followed with an F₀ between 1.03 and 1.53.

TABLE (Continued)

Run No.: PB-4
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: None
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation	Toxin
Can 1, 1.82	1	-	
Can 2, 1.82	2	-	
	3	-	
	4	-	
Can 1, 1.10	5	12	2/2
	6	13	
	7	10	
	8	15	
Can 1, 1.39	9	15	2/2
Can 2, 1.39	10	-	
	11	-	
	12	12	2/2

Conclusion: Under these conditions, canned green peas were sterilized by F₀ values between 1.39 and 1.82.

TABLE (Continued)

Run No.: PB-5
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spore Per Can
 Irradiation: 0.930 megarad
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation	Toxin
Can 1, 1.06	1	-	
Can 2, 1.06	2	-	
Can 3, 1.06	3	-	
	4	-	
Can 1, 0.86	5	-	
Can 2, 0.86	6	-	
Can 3, 0.86	7	-	
	8	-	
Can 1, 0.36	9	17	
Can 2, 0.36	10	20	
Can 3, 0.36	11	19	
	12	17	
Can 1, 0.50	13	-	
Can 2, 0.50	14	-	
Can 3, 0.50	15	-	
	16	-	

Conclusion: Under these conditions, canned green peas were sterilized by 0.930 megarad of gamma radiation followed with a F₀ between 0.36 and 0.50.

TABLE (Continued)

Run No.: PB-6
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: As Indicated
 Processing Temperature: Not Heat Processed
 Incubation Temperature: 85°F

Megarad	Can No.	Days-to-gas Formation
2.330	1	12
	2	18
	3	12
	4	19
2.790	5	-
	6	-
	7	-
	8	-
1.980	9	12
	10	17
	11	18
	12	10

Conclusion: Under these conditions, canned green peas were sterilized by between 2.33 and 2.79 megarad of gamma radiation.

TABLE (Continued)

Run No.: PB-7
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: None
 Irradiation: None
 Processing Temperature: 230°F
 Incubation Temperature: 85°F
 Object: To determine the F_0 required to sterilize canned green peas. The peas were frozen green peas purchased from The University of Michigan food stores.

F_0	Can No.	Days-to-gas Formation	Toxin
Can 1, 0.03	1	4	
Can 2, 0.03	2	5	
Can 3, 0.03	3	4	
	4	5	
Can 1, 0.06	5	4	0/2
Can 2, 0.06	6	6	
Can 3, 0.06	7	4	
	8	-	
Noninoculated, unheated control	9	3	0/2
Inoculated unheated control	10	3	2/2
Irradiated control, 0.465 megarad	11	11	
	12	11	

Conclusion: Canned green peas contain sufficient anaerobic bacterial spores in the frozen condition, as received by us, to require an F_0 greater than 0.06 or irradiation with more than 0.465 megarad, to produce sterile peas.

TABLE (Continued)

Run No.: PB-8
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: 1.395 Megarad
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation
Can 1, 0.48	9	-
Can 2, 0.48	10	-
Can 3, 0.48	11	-
	12	-
Can 1, 0.33	13	-
Can 2, 0.33	14	-
Can 3, 0.33	15	*32
	16	-
Can 1, 0.21	17	25
Can 2, 0.21	18	25
	19	30
	20	27
Can 1, 0.12	21	27
Can 2, 0.12	22	30
	23	25
	24	25
Can 1, 0.04	25	19
Can 2, 0.04	26	13
	27	18
	28	13

Conclusion: Under these conditions, canned green peas were sterilized by 1.395 megarad of gamma radiation followed with F₀ between 0.33 and 0.48.

*Positive for Type B Botulinum toxin.

TABLE (Continued)

Run No.: PB-9
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: None
 Irradiation: None
 Processing Temperature: 230°F
 Incubation Temperature: 85°F
 Object: Same as Run No. PB-7, to determine the F₀ required to sterilize noninoculated peas that we are using for our experiments.

F ₀	Can No.	Days-to-gas Formation	Toxin
Can 1, 0.027	1	3	
Can 2, 0.027	2	5	
	3	5	
	4	5	
Can 1, 0.036	5	5	
Can 2, 0.036	6	5	
	7	3	
	8	3	
Can 1, 0.036	9	5	
Can 2, 0.036	10	5	
	11	11	
	12	5	0/4
Can 1, 0.054	13	5	4/4
Can 2, 0.054	14	5	*4/4
	15	3	1/4
	16	-	

*Tests as Botulinum toxin type B with antiserum neutralization.

Conclusion: An F₀ in excess of 0.054 is needed to sterilize the non-inoculated canned green peas that we are using in our experiments.

TABLE (Continued)

Run No.: PB-10
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: As Indicated
 Processing Temperature: None
 Incubation Temperature: 85°F

Megarad	Can No.	Days-to-gas Formation
1.860	1	12
	2	11
	3	11
0.930	5	10
	6	7
	7	10
2.790	9	-
	10	22
	11	-

Conclusion: Under these conditions, slightly more than 2.790 megarad of gamma radiation are required to sterilize canned green peas.

TABLE (Continued)

Run No.: PB-11
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: None
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation
Can 1, 2.03	5	-
Can 2, 2.03	6	-
	7	-
	8	-
Can 1, 1.54	1	-
Can 2, 1.54	2	-
	3	-
	4	-

Conclusion: Under these conditions, canned green peas require an F₀ less than 1.54 to produce sterility.

TABLE (Continued)

Run No.: PB-12
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: None
 Irradiation: None
 Processing Temperature: 230°F
 Incubation Temperature: 85°F
 Object: Same as Runs PB-7 and PB-9, to determine the F_0 required to sterilize noninoculated peas that we are using for our experiments.

F_0	Can No.	Days-to-gas Formation
Can 1, 0.18	1	49
Can 2, 0.18	2	19
	3	5
	4	3
Can 1, 0.31	5	12
Can 2, 0.31	6	12
	7	16
	8	16
Can 1, 0.17	9	15
Can 2, 0.17	10	7
	11	7
	12	46
Can 1, 0.35	13	-
Can 2, 0.35	14	-
	15	12
	16	16
Can 1, 0.52	17	-
Can 2, 0.52	18	-
	19	-
	20	-
Can 1, 0.76	21	-
Can 2, 0.76	22	-
	23	-
	24	-
Can 1, 1.22	25	-
Can 2, 1.22	26	-
	27	-
	28	-

Conclusion: Under these conditions, canned green peas were sterilized by an F_0 between 0.35 and 0.52. This represents a rather considerable resistance to heat processing by the noninoculated (but naturally contaminated) peas which we used in these experiments.

TABLE (Continued)

Run No.: PB-13
 Can Size: No. 1 Picnic (211 x 400)
 Product: Green Peas
 Inoculum: None
 Irradiation: None
 Processing Temperature: 230°F
 Incubation Temperature: 85°F
 Object: Same as Runs PB-7, PB-9 and PB-12, to determine the F_0 required to sterilize the noninoculated peas we are using in our experiments.

F_0	Can No.	Days-to-gas Formation
Can 1, 0.041	1	3
Can 2, 0.041	2	3
	3	3
	4	3
Can 1, 0.20	5	-
Can 2, 0.20	6	8
	7	11
	8	20

Conclusion: An F_0 greater than 0.20 is needed to sterilize the canned peas "as received."

TABLE (Continued)

Run No.: PB-14
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: 0.465 Megarad
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation
Can 1, 0.60	1	25
Can 2, 0.60	2	29
Can 3, 0.60	3	18
	4	21
Can 1, 0.75	5	-
Can 2, 0.75	6	-
Can 3, 0.75	7	-
	8	-
Can 1, 1.10	9	-
Can 2, 1.10	10	-
	11	-
	12	-
Can 1, 0.50	13	-
Can 2, 0.50	14	-
Can 3, 0.50	15	21
	16	18

Conclusion: Under these conditions, canned green peas were sterilized by 0.465 megarad of gamma radiation followed with an F₀ between 0.60 and 0.75.

TABLE (Continued)

Run No.: PB-15
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: 0.650 Megarad
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation
Can 1, 0.92	1	-
Can 2, 0.92	2	-
	3	-
	4	-
Can 1, 1.12	5	-
Can 2, 1.12	6	-
	7	-
	8	-
Can 1, 0.80	9	22
Can 2, 0.80	10	-
	11	-
	12	-
<u>Controls:</u>		
Can 1, 0.50	13	8
Can 2, 0.50	14	8
Inoculated, nonirradiated	15	8
	16	8
Can 1, 1.10	17	-
Can 2, 1.10	18	-
Noninoculated, nonirradiated	19	-
	20	-
Can 1, 0.10	21	4
Can 2, 0.10	22	4
Noninoculated, nonirradiated	23	4
	24	4
Can 1, 0.39	25	8
Can 2, 0.39	26	8
Inoculated, nonirradiated	27	8
	28	8
Irradiated only (0.650 megarad)	1	4
	2	-
Inoculated	1	4
Noninoculated	1	4

Conclusions: (a) Under these conditions, canned green peas were sterilized by 0.650 megarad of gamma radiation followed by an F₀ between 0.80 and 0.92. (b) The nonirradiated, noninoculated control cans required an F₀ between 0.10 and 1.10 for sterilization. (c) An irradiation dose in excess of 0.650 megarad was needed to sterilize the noninoculated canned peas.

TABLE (Continued)

Run No.: PB-17
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: 1.96 Megarad
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation
Noninoculated control	a	4
	b	-
Inoculated control	A	6
Can 1, 0.33	1	-
Can 2, 0.33	2	-
Can 3, 0.33	3	-
	4	-
Can 1, 0.22	5	-
Can 2, 0.22	6	-
Can 3, 0.22	7	-
	8	-
Can 1, 0.11	9	-
Can 2, 0.11	10	-
Can 3, 0.11	11	-
	12	-
Can 1, 0.06	13	-
Can 2, 0.06	14	-
Can 3, 0.06	15	-
	16	-

Conclusion: Under these conditions, and after one month's incubation, canned green peas were sterilized by 1.96 megarad of gamma radiation followed by an F₀ less than 0.06.

TABLE (Continued)

Run No.: PB-18
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: 1.63 Megarad
 Processing Temperature: 230°F
 Incubation Temperature: 85°F

F ₀	Can No.	Days-to-gas Formation
Controls—same as on Run No. PB-17		
Can No. 1, 0.64	1	-
Can No. 2, 0.64	2	-
Can No. 3, 0.64	3	-
	4	-
Can No. 1, 0.30	5	-
Can No. 2, 0.30	6	-
Can No. 3, 0.30	7	-
	8	-

Conclusions: Under these conditions, and after one month's incubation, canned green peas were sterilized by 1.63 megarad of gamma radiation followed by an F₀ less than 0.30.

TABLE (Continued)

Run No.: PB-19
 Product: Green Peas
 Inoculum: 5,000,000 C. botulinum 213B Spores Per Can
 Irradiation: As Indicated
 Processing Temperature: None
 Incubation Temperature: 85°F

Megarad	Can No.	Days-to-gas Formation
Inoculated control	17	6
Noninoculated controls	NI	4
	NI	4
2.79	1	28
	2	-
	3	28
	4	-
3.29	5	-
	6	-
	7	-
	8	-
3.76	9	-
	10	-
	11	-
	12	-
2.35	13	28
	14	23
	15	23
	16	23

Conclusions: Under these conditions, and after one month's incubation, canned green peas were sterilized by between 2.79 and 3.29 megarad of gamma radiation.

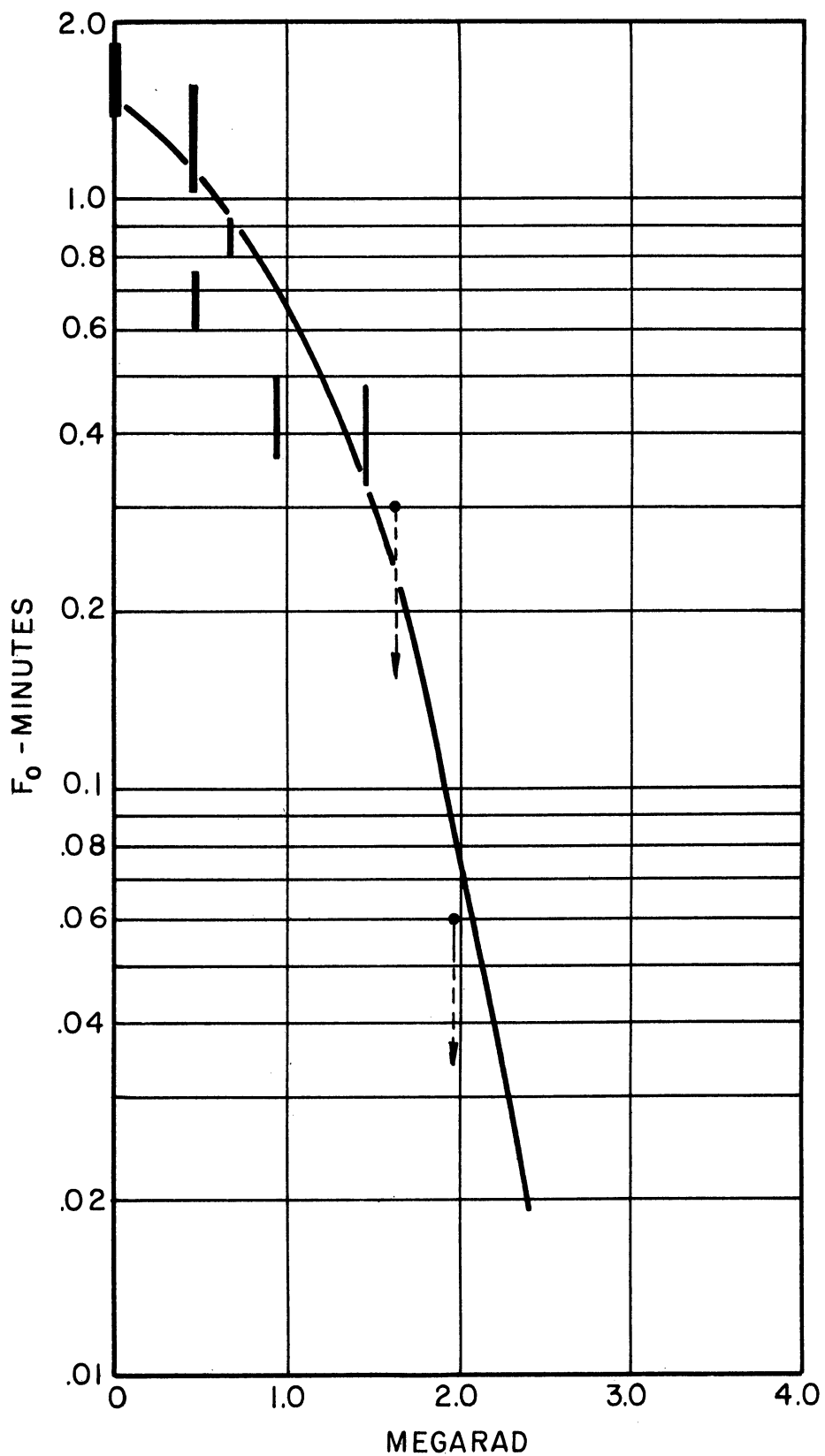


Fig. 1. F_0 required to sterilize green peas packed in No. 1 Picnic tin cans, inoculated with 5,000,000 *C. botulinum* 213B spores per can, and irradiated with gamma rays from Cobalt-60 before heat processing at 230°F and incubating at 85°F.

PHASE II

DETERMINATION OF RADIATION STERILIZATION DOSE FOR CANNED MEAT

Five experiments have been run and the cans are now incubating. One experiment was conducted with raw ground beef; the other four are at various C. botulinum 213B spore concentrations using cooked ground beef in 202 x 202 cans. However, incubation has only been in progress for a few weeks, so it would be premature to report data for these experiments. Spore concentrations as high as 100,000 per gram have been included in the work as of this date.

PHASE III

DETERMINATION OF COMBINED IRRADIATION-HEAT PROCESSING TREATMENTS REQUIRED TO STERILIZE CANNED MEAT PRODUCTS

Eight experiments utilizing "Savortite" Pure Pork Luncheon Meat, furnished by Swift and Co., have been run. To date only the controls have developed gas, so it would also be premature to report these data. Since long incubation periods are apparently going to be needed before results are available, the eight runs were made at various combined irradiation-heat processing treatments that, in our judgment, would yield information regarding suitable treatments. The validity of our estimates will be determined only by observations after a few more months of incubation. Therefore evaluation of results may be delayed for some months.

At present we are continuing Phase II of the work, working principally with C. botulinum 213B in cooked meat. When this series is in the incubator, we will work on the raw meat.

Our Cobalt-60 source is to be modified soon. This may interrupt some of the irradiation work for a couple of weeks; this should give us time to collect more spores.

STATEMENT OF PROGRESS

(a) Approximately 40% of the contracted work is complete as of this date.

(b) About half of the budgeted \$25,000 have been spent as of this date.

(c) It is probable that the work can be completed within the budget, provided that a contract for continuation of work in this general area is again sponsored for us by the Quartermaster Corps. However, if such a contract is not forthcoming for next year, we will need to reduce expenses severely the last two months which represent extension of the time period, by the Quartermaster Corps, beyond our originally calculated expenditure schedule.

