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BIOLOGICAL AND ANIMAL CONTAINER STUDIES

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OF ATOMIC WEAPONS TEST

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Annex 12

BIOLOGICAL AND ANIMAL CONTAINER STUDIES

(SERVICE TEST NO. 7)

Preliminary Report

MIXED RADIATION EXPERIMENTS WITH MAIZE

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MIXED RADIATION EXPERIMENTS WITH MAIZE

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CONTENTS

INTRODUCTION

The Bikini Tests with maize seed demonstrated that the most heavily radiated sample that was recovered from the bombed area exhibited effects comparable to those induced by approximately 15,000 r-units of X radiation. Tests conducted in 1948, which included a similar comparison of samples received from the Navy on May 24, 1948 and other samples irradiated with known doses of X rays, have shown that the samples received from the Navy had been exposed to a wide range of intensities of ionizing radiations. These radiations induced effects comparable to the effects induced by doses of X rays ranging from less than 5,000 r to more than 120,000 r.

In this preliminary report the injurious effects of the radiation on the seedlings and plants produced by the irradiated seed are described and illustrated.

EXPERIMENTAL DESIGN

The plan of the experiments involved a comparison of the effects induced in seed treated with measured doses of X rays and other samples of the same seed received from the Navy and hereafter referred to as "mixed radiation" samples. These samples had been exposed to ionizing radiation of unknown quality and intensity. X-ray dosages of 5, 10, 20, 30, 40, 60, 90 and 120 thousand r-units were applied to samples of seed April 15 and 16, 1948. These dosages were applied at the rate of from 90 to 140 r per minute of unfiltered radiation at distances of 40 to 60 cm from the tungsten target. Germination trials of the X-rayed and mixed radiation samples, received at Ithaca, N.Y. on May 24, were made immediately thereafter for the purpose of selecting seed lots for field plantings and to record the effects of the radiation on germination and seedling growth. Additional germination trials were made later for further comparison of radiation effects in the seedling stage.

Various injurious effects appeared as the seed germinated and the seminal roots and seedling leaves developed. These effects in the order of their appearance as the dose was increased from low to high intensities were: (1) a mottling of the seedling leaves; (2) reduction in rate of top growth; (3) cessation of top growth at the coleoptile stage and the subsequent death of the seedlings; (4) reduction in rate of seminal root growth; and (5) failure of emergence due to cessation of growth in the initial stages of germination.

Comparison of these effects in the mixed radiation samples and in the X-rayed samples which had received measured doses of ionizing radiations ranging from 5,000 to 120,000 r provided a technique for estimating the amounts of radiation to which the mixed samples had been exposed.

The seedling trials were made with the dent corn hybrid L 289/I 205, and with a stock of tetraploid dent corn to determine the relative sensitivity of diploid and tetraploid corn to the radiations. The seed was germinated under greenhouse conditions in sand flats for the measurement of seedling growth rates and in incubator trays for the measurements of seminal root growth.

RESULTS

MOTTLING EFFECTS

A mottled appearance of the seedling leaves develops as a result of exposure to the lower doses of radiations involved in these experiments. This mottling effect is due to the failure of chlorophyll to develop in areas which were from 1 to 2 mm in diameter and in length ranged from 2 to 3 mm in the first seedling leaf to 8 mm or more in second and third seedling leaves. Apparently these chlorotic spots and streaks originated from individual irradiated cells which subsequently divided to form more or less elongate multicellular areas of chlorophyll-deficient tissue.

An X-ray dose of 5,000 r caused a light mottling of the seedling leaves. The controls showed no mottling and none was apparent in the mixed radiation samples 8 to 10 and 15. Doses of 10,000 r caused more pronounced mottling and at 20,000 r and higher doses the occasional seedling leaves that developed were very heavily mottled, too heavy in fact for dosage comparisons. The mixed sample No. 7 was faintly mottled, showing showing somewhat less injury than the 5,000-r sample; No. 6 was as heavily mottled as the 10,000-r sample; No. 14 was comparable to the 20,000-r sample and No. 5 had a mottled appearance intermediate between that of the 10,000- and 20,000-r samples, (c.f. Fig. 1). The seedlings obtained from mixed samples 1 to 4 and 12 and those from X-ray dosages ranging from 20,000 r to 120,000 r rarely grew beyond the coleoptile stage. The occasional seedling leaves that did develop in these cultures were chlorotic and unsuited for estimating the amount of mottling. From these comparisons it appeared that the mixed radiation samples 8 to 10 and 15 received appreciably less than 5,000-r units, sample 7 slightly less than 5,000 r, No. 6 about 10,000 r, No. 5 between 10,000 and 20,000 r and No. 14 about 20,000 r. Samples 1 to 4 and 12 were so heavily mottled and deficient in growth that it was only apparent that they had received doses of 30,000 r or higher. The amount of radiation these samples received was more accurately estimated from tests of relative growth rates of the tops and roots.

REDUCTION IN TOP GROWTH OF SEEDLINGS

The amount of top growth in the seedling stage was determined by measuring the height of the tallest seedling leaf 7 days after planting, and again 5 days later to determine growth increments during the interval from 7 to 12 days. The first planting on May 24 consisted of approximately 20 seeds of each of the X-rayed and mixed radiation samples of the diploid dent hybrid L 289/I 205 and of the mixed radiation samples of the tetraploid dent. A second planting of 30 seeds of each lot was made on July 10. Observations on the appearance of the seedlings and growth measurements were taken 7 and 12 days after the seeds were planted. Photographs of representative seedlings from the X-rayed and mixed radiation samples are reproduced in Figs. 2, 3, and 4. The measurements are recorded in Tables 1 to 12. The average height of each lot of seedlings and the per cent of

Fig. 1a Mottling of seedling leaves induced by 5,000-, 10,000-, and 20,000-r X-ray dosages. Control at right.

Fig. 1b Mottling of seedling leaves in mixed radiation samples 7,6,5,14. Control at right.

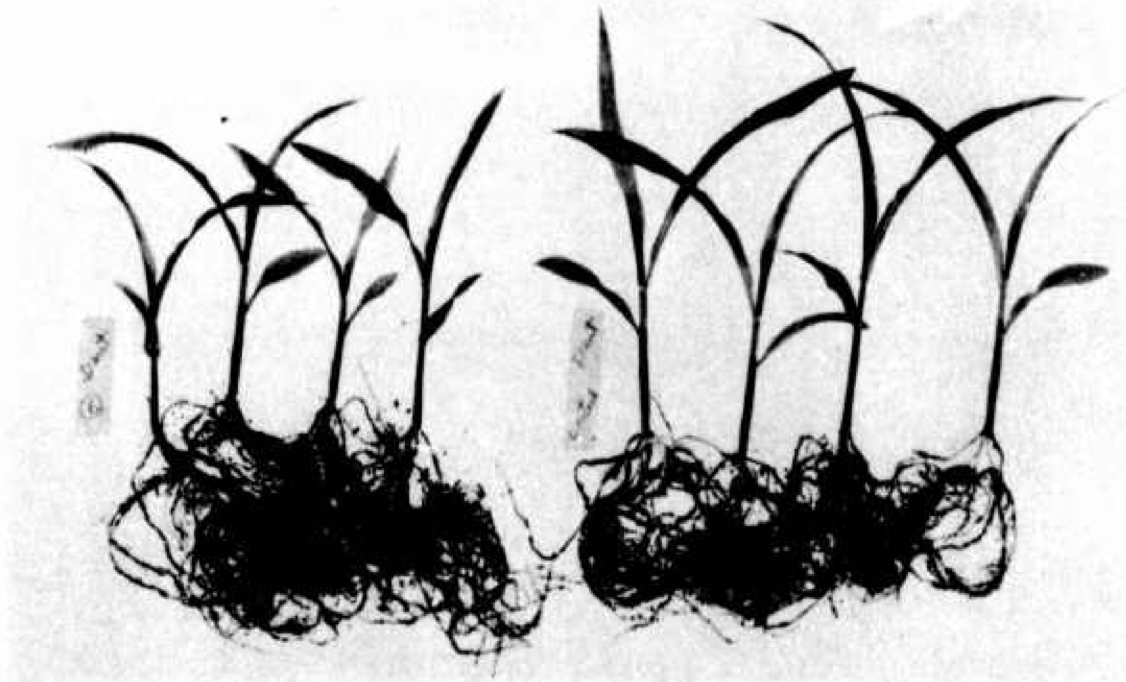

Fig. 2a Seedlings of mixed radiation sample 6 and control.

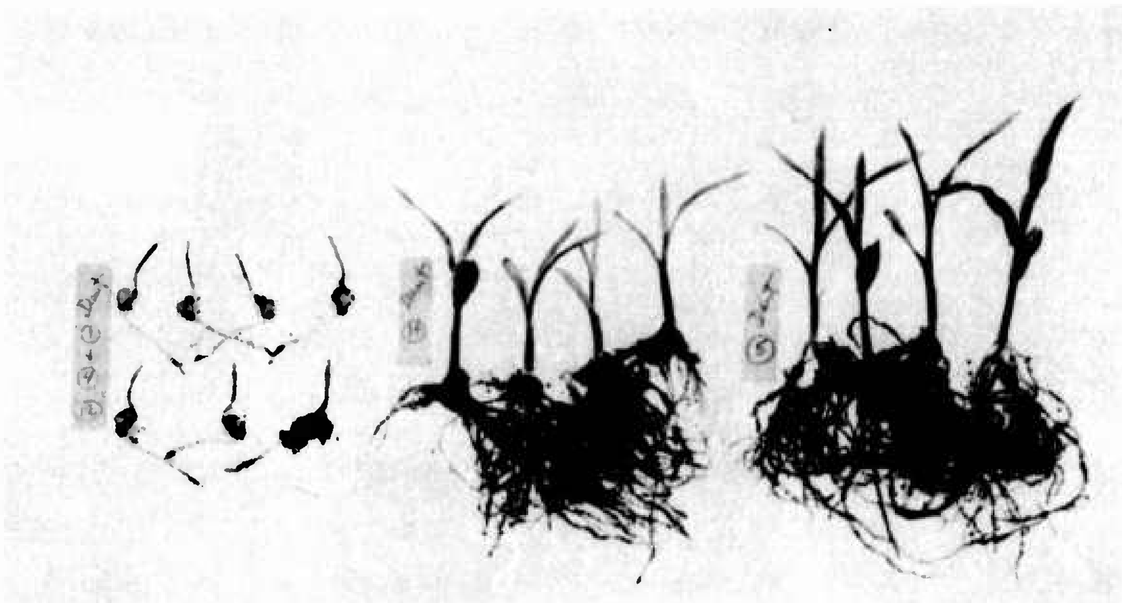

Fig. 2b Seedlings from most severely injured mixed radiation samples

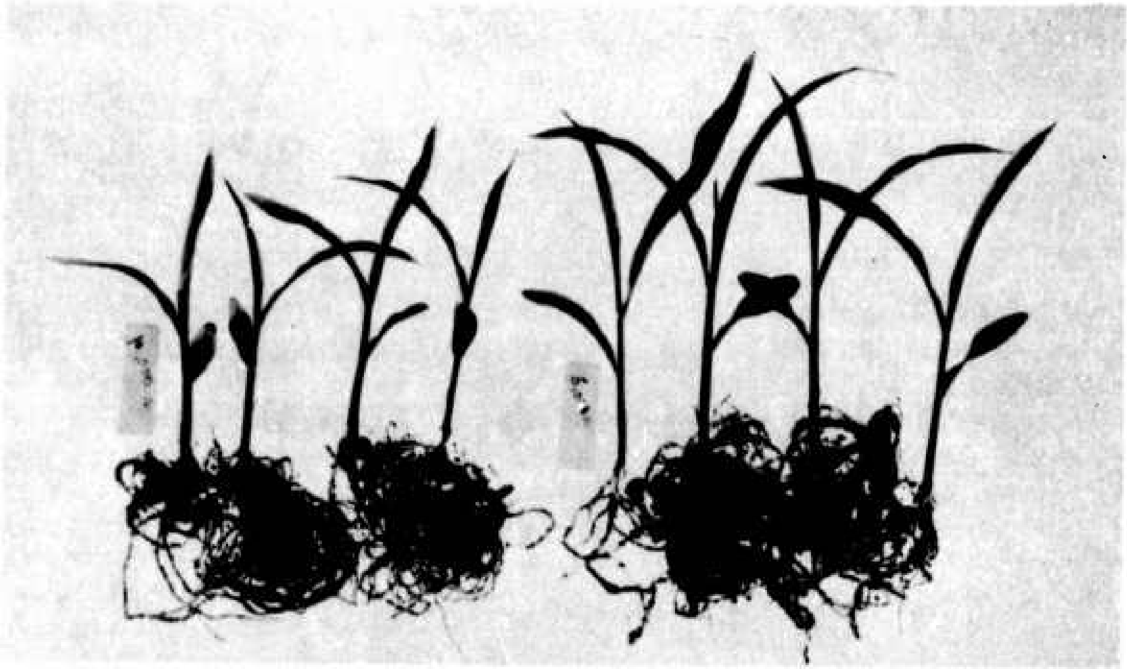

Fig. 3a Seedlings from X-ray dosages of 5,000 r and 10,000 r.

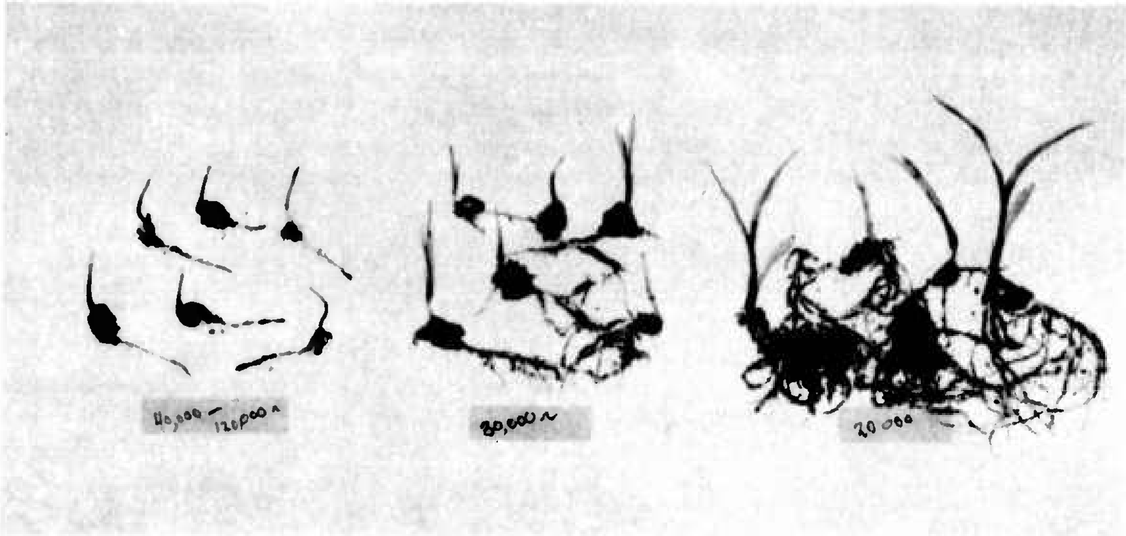

Fig. 3b Seedlings from X-ray dosages of 20,000 r to 120,000 r.

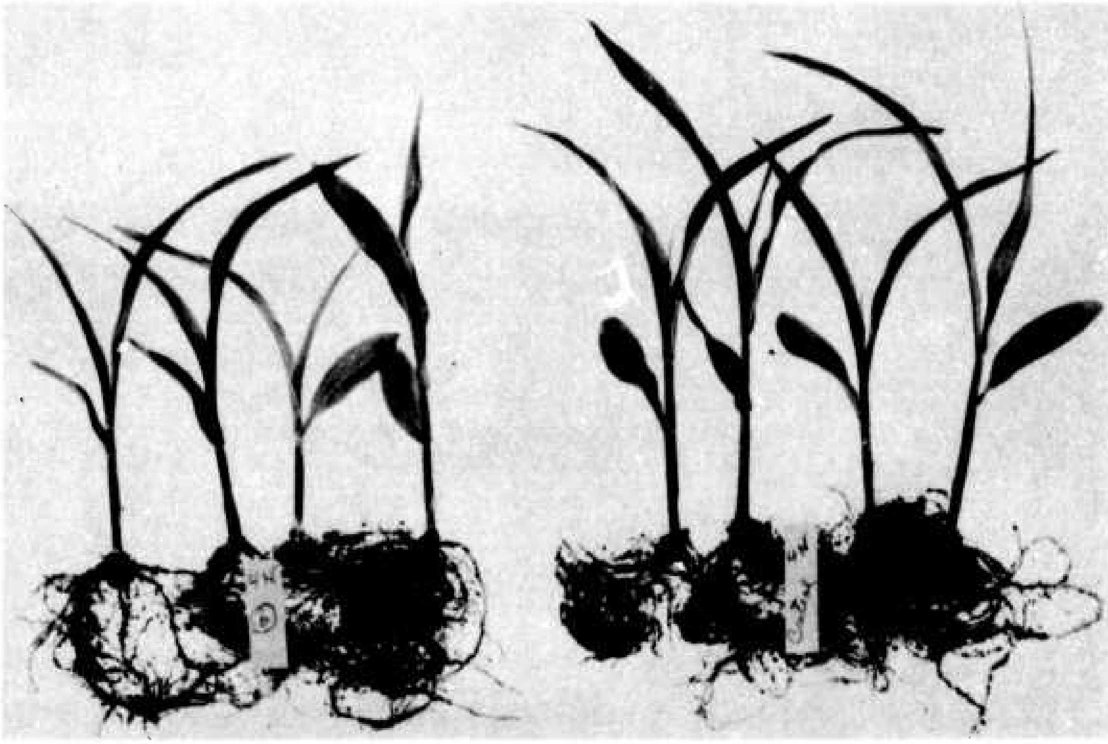

Fig. 4a Seedlings of tetraploid corn from mixed sample 6 and control.

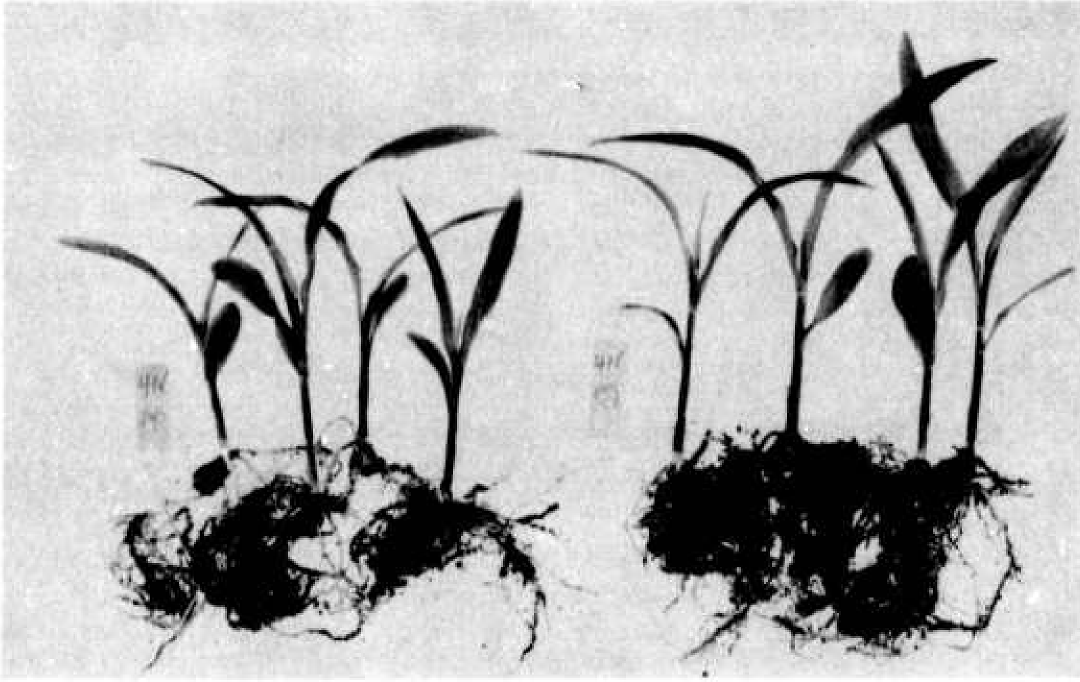

Fig. 4b Seedlings from tetraploid corn exposed to mixed radiation samples 5 and 14.

normal growth, the controls being taken as 100 per cent, were computed for each sample. The measurements were recorded in millimeters and it was estimated that the sampling error was not more than 3 per cent.

The seedlings which were given X-ray doses of 5, 10, 20, 30, 40, 60, 90 and 120 thousand r-units showed decreasing percentages of normal growth of 97, 71, 33, 23, 17, 14, 12 and 12 per cent (Table 1 and Fig. 2). The amount of growth decreased appreciably with increasing dosages up to 60,000 r; but there was not much further reduction thereafter. This was due to a cessation of growth in the coleoptile stage at the higher doses. During the next 5 days the seedlings of the untreated control more than doubled their height, those given weaker doses of X rays, from 5,000 to 30,000 r, increased appreciably in height but there was little or no increase in height of the more heavily X-rayed samples given from 40,000 to 120,000 r (Table 2 and Fig. 3).

The mixed radiation samples 8 to 10 and 15 showed in comparison with the control little or no significant difference in growth. Samples 7, 6, 5, 14 exhibited 89, 59, 51 and 41 per cent of normal growth; samples 1 to 4 and 12 exhibited amounts of growth ranging from 11.6 and 17 per cent of normal (Table 3). The growth of most of the seedlings in these more heavily radiated samples stopped at the coleoptile stage, the coleoptile ordinarily developing to a height of about 8 to 15 mm above the soil level. There was also a failure of emergence of a limited number of seedlings from the more heavily irradiated samples.

The seedlings of the tetraploid dent corn were less severely injured by the mixed radiation than were those of the diploid dent hybrid; heavier doses were required to produce similar effects due presumably to the presence of the two extra sets of chromosomes which would tend to protect the plant from the injurious effects of chromosomal mutations. These differences are shown in Tables 3 and 4 and in Figs. 2 and 4.

CESSATION OF TOP GROWTH AT THE COLEOPTILE STAGE

Seedling growth from the diploid lots of seeds that had received X-ray doses ranging from 30,000 to 120,000 r or equivalent doses of mixed radiation rarely continued beyond the coleoptile stage. Growth up to this stage was completed, or nearly so, within a week after the seeds were planted and very little growth occurred thereafter either in the samples that received 30,000 or more roentgens of X rays or in mixed samples 1 to 4 and 12. There was no significant increase in the average height of the seedlings in these cultures during the period from 7 to 12 days after planting, when very appreciable increases occurred in the less heavily irradiated samples (compare Table 1 with Table 2 and Table 3 with Table 4). All of the measurements in the coleoptile and other stages of post emergent seedling growth were taken from the soil level. Measured in this manner the coleoptiles ranged in height from about 5 to 35 mm. The lethal effect of these heavy dosages caused the death of the seedlings usually within ten days or two weeks after planting.

At a somewhat later stage, usually following the partial emergence of one or two leaves from the coleoptile, the seedlings in the 20,000 r and mixed radiation samples 5 and 14 succumbed to the delayed killing action of the radiations.

The seed of the tetraploid dent corn tolerated somewhat heavier doses of the ionizing radiations. There was less reduction in growth at sublethal dosages and many of the plants in mixed sample 14 grew to maturity. These differences in the response of the diploid and tetraploid seed to the radiations are apparent from comparisons of seedling growth rates in Tables 1 to 4 and 5 to 6; also Tables 7 to 10 and 11 to 12 which are from a duplicate set of seedling comparisons. Photographs of diploid and tetraploid seedlings of the same age from the mixed radiation samples are shown in Figs. 2 and 4.

FAILURE OF SEEDLING EMERGENCE

The heaviest doses included in these experiments inhibited the growth, of a limited number of seedlings prior to emergence or as the coleoptiles were protruding slightly above the soil level. Within the dosage range from 60,000 to 120,000 r, approximately one-third to one-half of the seedlings either failed to emerge or ceased growth before they extended more than a few millimeters above the soil. Within the range of variability in the very limited amounts of seedling growth produced by the most heavily irradiated seeds it was not possible with the numbers of individuals involved in these tests to correlate the amount of growth with dosages from 40,000 to 120,000 r.

The highest X-ray dose of 120,000 r was not sufficient to inhibit germination of the seed. Plumules several millimeters in length and appreciably longer primary seminal roots were produced by the treated seed before the lethal action of the treatments was apparent. The mixed radiation samples 3 to 4 and 12 were similarly affected.

RETARDATION OF SEMINAL ROOT GROWTH

The germination trials of seed planted in sand flats described above, indicated that the amount of seminal root growth was appreciably less at the higher dosages than at somewhat lower dosages. To explore the possibility that root growth might furnish a criterion for distinguishing between dosages at the higher levels, germination trials were conducted by placing the seeds between moist paper toweling in a germinator maintained at a uniform temperature of 28° to 29° C. Measurements of root growth were made 3 days after the seed was placed in the germinator. At this stage the controls had primary seminal roots from 5 to 7 cm in length and secondary seminal roots up to 2.5 cm in length.

Mixed samples 6 to 10 and 15 and X-rayed samples from 5 to 20,000 r had as much root growth as the controls. Retardation of seminal root growth was apparent at X-ray dosages of 30 and 40,000 r and in the mixed radiation sample 5. There was more pronounced retardation at 60 and 90,000 r and in mixed samples 3, 4 and 14. At 120,000 r and in the mixed samples 1, 2 and 12, seedling root growth was limited to the development of the primary seminal root and an occasional rudimentary secondary seminal root, the greatest amount of reduction being in sample No. 1. These comparisons are summarized in the following table.

Maximum Root Growth in cm		X-ray Dosages in thousands of r-units	Mixed Radiation Samples
Primaries	Secondaries		
		Controls and dosages	Controls and
7	2.5	of 5, 10, 20	samples 6-10, 15
5.5	2.0	30, 40, 60	5, 14
4.5	1.5	90	3, 4
3.5	1.0	120	1, 2, 12

Thus the retardation of seminal root growth following heavy doses of X rays from 60 to 120 thousand r-units provided a means of estimating the amount of radiation received by the more heavily irradiated mixed samples. It was not possible to differentiate definitely between samples 1, 2 and 12 as there was very little seminal root growth in any of these samples. The possibility exists that at least one of these three samples received an equivalent of appreciably more than 120,000 r of X radiation: sample 1 appeared to have produced less seedling growth than the X-rayed sample exposed to 120,000 r.

FIELD COMPARISONS OF PLANT GROWTH

The performance under field conditions of plants grown to maturity from X-rayed samples given 5,000 and 10,000 r is illustrated in Fig. 5, and in Fig. 6 are shown plants from mixed samples 5 and 6. There are five rows of plants in each lot with adjacent rows of untreated plants on each side of the five-row blocks. The photographs were all taken on the same day just at the close of the sporocyte season. Fig. 7 illustrates the more vigorous growth of the mixed radiation samples

Fig. 5a Center five rows X-rayed 5,000 r; adjoining rows untreated controls.

Fig. 5b Center five rows X-rayed 10,000 r; adjoining rows untreated controls.

Fig. 6a Center five rows mixed radiation sample 5; adjoining rows untreated controls.

Fig. 6b Center five rows mixed radiation sample 6; adjoining rows untreated controls.

Fig. 7a Center five rows tetraploid plants from mixed radiation sample 5; adjoining rows untreated controls.

Fig. 7b Center five rows tetraploid plants from mixed radiation sample 6; adjoining rows untreated controls.

Nos. 5 and 6 of the tetraploid. In Fig. 8 the extremely defective appearance of tetraploids from mixed sample 14 is apparent especially in the close-up photograph (Fig. 8b). None of the plants in the comparable diploid sample 14 survived the seedling stage.

CONCLUSIONS

The mixed radiation samples of corn seed received from the Navy May 24, 1948 were compared with other samples of the same seed that were irradiated with doses of X rays ranging from 5,000 r to 120,000 r. Radiation effects included mottling of the seedling leaves, retardation of seedling top growth, cessation of seedling growth in the coleoptile stage, failure of emergence and retardation of seminal root growth.

An X-ray dose of 5,000 r caused a slight mottling of the seedling leaves, but relatively little retardation in the growth of the seedlings. 10,000 r caused pronounced mottling and an appreciable retardation of seedling growth but no appreciable killing effect in the seedling stage. 20,000 r caused heavy mottling and none of the plants survived the seedling stage. Dosages of 30,000 to 120,000 r were lethal in the coleoptile stage or in pre-emergence stages. Seminal root growth was inhibited appreciably at dosages of 30,000 r and higher, the amount of inhibition increasing at the higher dosages.

From comparisons of these radiation effects in the X-rayed and mixed radiation samples it was concluded that the mixed samples 8 to 10 and 15 received an equivalent of less than 5,000 r, sample 7 slightly less than 5,000 r, sample 6 approximately 10,000 r, sample 5 about 15,000 r,

Fig. 8a Center five rows are tetraploid plants from mixed radiation sample 14; adjacent rows untreated controls.

Fig. 8b Close-up view of deformed plants of mixed radiation sample 14.

sample 14 about 20,000 r. Samples 3 and 4 received appreciably heavier dosages, possibly as high as 60 to 90,000 r, and the limited seedling growth of samples 2 and 12 was comparable to that of X-rayed lots of seed given 120,000 r. The more pronounced inhibition of seedling growth in mixed sample No. 1 indicated that it may have received appreciably more than the equivalent of 120,000 r.

Thus these mixed samples received a maximum of at least 10 times as much ionizing radiations as the most heavily irradiated Bikini samples that were recovered from the target area and subjected to comparable tests.

TABLE 1

Seedling Measurements of X-rayed Diploid Dent Hybrid

Series 1a, Measured June 1, 1948

Control		X-ray Dosage in Units of 1,000 r							
		5	10	20	30	40	60	90	120
	98	103	60	32	28	19	15	8	9
	108	75	83	30	20	20	15	8	15
	99	114	91	40	33	15	18	11	8
	78	99	52	36	16	6	17	14	9
	107	97	68	34	14	8	12	22	9
	105	85	75	37	14	18	16	15	4
	105	82	79	33	16	9	15	18	18
	101	105	56	41	21	23	9	4	15
	73	82	84	21	22	19	11	8	16
	78	101	60	17	22	19	11	8	15
	98	102	93	34	25	15	12	14	15
	101	100	54	26	25	19	18		
	108	72	72	40	21	21			
	103	106	42	35	18	16			
	103	103	77	34	38	22			
Total	1465	1426	1046	490	333	249	169	130	133
Average	97.6	95.0	69.7	32.6	22.2	16.6	14.0	11.8	12.0
% Normal	100	97	71	33	23	17	14	12	12

TABLE 2

Seedling Measurements of X-rayed Diploid Dent Hybrid

Series 1b, Measured June 6, 1948

Control		X-ray Dosage in Units of 1,000 r							
		5	10	20	30	40	60	90	120
	195	188	155	60	79	23	23	17	15
	185	198	144	68	41	16	15	15	15
	197	194	92	50	35	20	13	12	14
	202	188	178	21	17	17	10	21	14
	187	195	118	51	20	12	8	15	16
	176	178	182	30	16	16	13	24	8
	160	195	145	21	20	17	14	16	11
	210	177	164	82	19	21	13	12	13
	206	180	159	89	22	11	15	10	9
	216	192	139	60	22	20	19	11	17
	177	195	91	68	16	10	17		9
	187	228	172	70	14	6	14		
	225	177	152	47	13	14			
	216	206	128	41	15	19			
					23	17			
Total	2739	2691	2019	758	372	239	174	153	141
Average	195.6	192.2	144.2	54.1	24.8	15.9	14.5	15.3	12.8
% Normal	100	98	74	28	13	8	7	8	6

TABLE 3

Seedling Measurements of Mixed Radiation Lots of Diploid Dent Hybrid
Series 1a, Measured June 1, 1948

	Control	Lot Numbers of Samples												
		1	2	3	4	5	6	7	8	9	10	12	14	15
	98	22	7	15	15	58	68	92	80	110	73	11	56	93
	108	18	19	15	14	52	27	80	112	108	110	16	27	85
	99	21	5	11	8	40	68	80	87	102	105	16	38	94
	78	15	9	20	4	58	58	79	98	108	97	15	42	89
	107	10	17	14	11	50	79	107	103	106	102	21	36	95
	105	18	13	20	12	50	52	85	105	107	110	20	45	87
	105	17	13	20	14	54	62	81	106	121	107	20	46	76
	101	6	7	17	15	65	51	88	110	97	96	20	25	90
	73	15	12	10	9	48	56	98	89	101	91	15	49	91
	78	21	14	14	4	24	51	94	102	106	88	22	41	91
	98	5	14	7	7	55	68	87	97	105	100	14	40	97
	101	12	8	5	12	48	67	98	92	107	100	16	54	102
	108	15	14	18	8	48	81	75	100	107	106	16	32	101
	103	18	11		16	57	60	102	102	112	92	17	32	98
	103	11				63	35	93	88	112	98	16	50	110
Total	1465	224	163	186	185	770	883	1339	1471	1609	1475	255	613	1399
Average	97.6	14.9	11.6	14.3	13.2	51.3	58.8	89.2	98.0	10.72	98.3	17.0	40.8	93.2
% Normal	100	15	12	15	14	53	60	91	100	110	101	17	42	95

TABLE 4

Seedling Measurements of Mixed Radiation Lots of Diploid Dent Hybrid
Series lb, Measured June 6, 1948

	Control	Lot Numbers of Samples												
		1	2	3	4	5	6	7	8	9	10	12	14	15
	195	7	10	21	18	137	58	186	145	198	188	17	85	199
	185	17	15	7	10	110	130	189	179	194	170	16	36	192
	197	15	7	5	16	101	162	185	168	190	183	15	[illegible]8	185
	202	11	15	14	9	99	150	182	164	192	176	16	91	197
	187	6	16	10	4	117	142	184	180	188	187	12	67	184
	176	21	14	19	8	48	108	199	179	198	158	23	61	156
	160	16	11	18	15	110	118	178	186	196	192	15	90	189
	210	7	12	21	15	140	99	178	190	210	195	17	25	176
	206	20	14	14	15	116	121	203	180	214	197	21	92	158
	216	19	19	20	8	104	105	184	175	200	201	22	74	166
	177	11	12	13	7	108	144	173	177	186	195	20	55	174
	187	16	7	15	14	121	120	170	161	200	174	15	80	165
	225	22	20	16	14	87	156	208	164	186	185	18	68	193
	216	21	6	6		118	138		178	190	173	16	40	165
		23			133	133		17	179	184	138	12	115	183
Total	2739	232	178	199	153	1649	1751	2419	2605	2926	2712	255	1017	2682
Average	195.6	15.4	12.7	14.2	11.7	109.9	125.0	186.0	173.6	195.0	180.8	17.0	67.8	178.8
% Normal	100	8	6	7	6	56	64	95	89	99	92	9	35	91

TABLE 5

Seedling Measurements of Mixed Radiation Lots of Tetraploid Dent
Series Ia, Measured June 1, 1948

	Control	Lot Numbers of Samples												
		1	2	3	4	5	6	7	8	9	10	12	14	15
	120	20	30	25	36	72	71	96	118	90	103	5	105	78
	93	18	2	32	40	69	69	97	73	107	85	12	76	82
	102	21	8	22	35	83	88	100	110	110	88	28	62	78
	98	19	19	8	32	71	76	104	86	87	93	12	85	77
	105	20	24	20	34	93	77	80	85	95	97	18	84	52
	109	7	21	22	30	68	93	96	95	90	95	22	114	56
	93	21	15	12	32	76	82	95	108	95	102	27	75	89
	67	27	27	35	31	72	94	118	104	96	80	27	78	114
	85	22	37	33	34	70	70	88	107	108	86	22	54	99
	118	24	32	32	30	81	78	96	75	108	77	28	53	95
	84	20	22	32	36	26	101	70	102	102	106	16	75	101
	96	32	20	17	34	91	90	40	92	94	80		74	86
	125	29	17	31	29	105	84	70	105	105	112		62	108
	105		25	32	25		104	110	74	112	100		88	
	104						69		112		93		73	
Total	1504	280	289	353	458	977	1246	1260	1446	1399	1397	217	1158	1115
Average	100.2	21.5	20.6	25.2	32.7	75.1	83.0	90.0	96.4	99.9	93.1	19.7	77.2	85.7
% Normal	100	21	21	25	33	75	83	90	96	100	93	20	77	86

TABLE 6

Seedling Measurements of Mixed Radiation Lots of Tetraploid Dent Series lb, Measured June 6, 1948

	Control	Lot Numbers of Samples												
		1	2	3	4	5	6	7	8	9	10	12	14	1'
	211	30	17	35	28	212	157	225	230	230	225	16	145	230
	219	23	23	20	35	168	220	120	175	216	199	29	165	207
	255	26	31	34	36	42	185	82	217	211	196	22	157	185
	237	19	35	32	32	177	170	185	196	163	160	28	176	170
	203	23	23	25	32	164	168	204	218	190	203	27	148	181
	260	21	24	23	33	182	151	180	158	180	175	22	100	267
	205	19	20	22	38	177	206	236	235	202	186	20	122	205
	177	10	25	38	32	165	165	205	230	182	173	29	137	122
	180	19	17	33	34	184	156	193	225	170	191	17	176	116
	196	19	25	20	36	177	170	163	206	205	198	6	226	180
	186	9	20	32	37	84	191	184	190	174	172	7	166	168
	180	22	9	36	41	145	182	195	205	203	193		196	165
	214	20	32	8	29	138	187	201	215	208	196		150	162
	190	28		21	34		164	184	143	176	188		175	
	250	2					165		230		197		211	
Total	3163	288	301	379	477	2015	2637	2557	3073	2710	2627	223	2450	2358
Average	210.8	20.5	23.1	27.0	34.0	155.0	175.8	182.6	204.8	193.5	175.1	20.2	163.3	181.3
% Normal	100	10	11	13	16	74	83	87	97	92	83	10	77	86

TABLE 7

Seedling Measurements of X-rayed Diploid Hybrid

Series 2a Measured July 17, 1948

Control	X-ray Dosages in Units of 1.000 r							
	5	10	20	30	40	60	90	120
173	129	137	11	9	3	9	4	7
160	140	78	11	5	2	9	7	5
167	140	61	10	6	7	11	8	13
172	159	56	6	2	2	3	8	2
190	128	85	10	12	6	4	7	4
187	158	87	15	14	5	9	9	3
143	149	62	11	6	5	5	3	7
179	146	91	11	13	6	12	2	7
162	130	84	11	7	4	12	7	7
138	144	20	18	11	5	6	6	6
157	129	21	7	15	2	5	7	3
186	121	60	24	6	9	6	6	8
168	127	72	9	13	4	7	4	5
190	163	47	10	8	6	10	3	5
186	126	12	13	20	4	8	9	8
180	124	66	6	5	10	7	6	12
165	152	62	11	4		10	2	
209	109	82	3	12		4	2	
176	120	49	5	7		5	4	
182	65	56	14	13		2	5	
199	134	117	8	10			2	
131	140	70	2	7			3	
98	149	65		8			2	
153	129	17		4			6	
153	143	110		3			5	
173	119	73		6				
164	136	60		10				
182	198	92		14				
182	94	79						
179	156	40						
Total 5084	3957	2011	227	250	80	144	127	102
Average 169.4	131.9	67.0	10.3	8.9	5.0	7.2	5.08	6.3
% Normal 100	78	40	6	5	3	4	3	4

TABLE 8

Seedling Measurements of X-rayed Diploid Dent Hybrid

Series 2b, Measured July 22, 1948

Control	X-ray Dosages in Units of 1,000 r							
	5	10	20	30	40	60	90	120
190	202	207	20	13	2	8	5	9
222	223	136	12	5	4	9	9	5
207	234	89	11	7	7	10	9	14
232	218	81	9	13	7	2	3	2
292	217	43	6	4	6	3	8	4
269	205	155	8	8	4	9	2	14
172	209	159	15	14	5	5	7	8
245	208	110	10	7	4	13	5	8
227	189	140	9	12	5	12	10	4
182	231	135	9	15	8	8	5	7
209	198	20	19	7	6	3	4	5
284	227	19	6	13	3	8	3	7
242	198	94	27	6	12	6	9	6
298	234	123	9	15		9	6	7
238	194	62	10	7		6	4	9
292	181	117	11	3		7	2	10
244	231	93	5	11		9	3	
316	189	140	12	8		2	2	
231	197	89	6	14		3	2	
254	120	112	5	10		2	2	
295	196	184	14	5			7	
166	215	112	8	7			6	
149	219	109		6				
201	171	14		4				
219	202	199		5				
199	184	124		9				
225	167	92		15				
248	169	170						
268	155	140						
253	235	12						
Total 7069	6018	3280	241	243	73	134	113	119
Average 235.6	200.6	109.3	10.9	9.0	5.6	6.7	5.1	7.4
% Normal 100	85	46	5	4	2	3	2	3

TABLE 9

Seedling Measurements of Mixed Radiation Diploid Dent Hybrid
Series 2a, Measured July 17, 1948

	Control	Lot Numbers of Samples												
		1	2	3	4	5	6	7	8	9	10	12	14	15
	154	88	9	8	5	92	86	168	136	157	215	13	14	194
	160	10	10	3	10	29	87	139	155	192	193	6	35	156
	157	12	6	5	3	18	89	152	161	130	189	9	25	156
	168	12	6	6	6	52	26	161	135	58	157	7	18	152
	176	13	14	2	11	31	94	164	164	164	155	8	9	202
	168	13	8	5	5	22	37	164	139	142	139	9	14	134
	183	12	8	5	12	25	74	167	140	146	162	3	15	169
	167	3	8	3	10	64	22	79	166	188	133	7	16	174
	164	4	9	5	10	39	34	148	117	188	143	10	26	162
	183	8	2	9	14	17	45	89	135	140	143	5	10	168
	167	4	4	8	14	89	44	150	165	191	158	5	15	146
	182	9	7	8	7	62	40	150	160	179	181	10	41	147
	187	14	6	6	7	26	11	138	160	141	161	3	23	119
	189	5	6	7	8	23	8	129	134	182	174	4	13	174
	165	7	11	8	5	46	97	148	179	148	132	5	46	179
	180	4	9	9		26	12	160	168	142	171	9	17	151
	174	4	4	2		52	98	124	132	170	153	2	23	169
	189	9	9	7		46	109	113	117	115	145	3	15	145
	175	12	10			51	61	147	85	162	144	9	51	188
	180	10	7			70	32	148	156	154	132	3	7	163
	173	10	6			24	86	197	173	182	138	5	18	125
	183	7				13	99	117	145	114	161		9	157
	179	11				48	23	146	142	171	180		43	160
	179	10				51	31	115	157	154	172		23	170
	184	15				44	16	143	134	175	169		17	165
	188					52	98	116	142	169	156		2	156
	169					54	85	113	120	156	151		14	145
	169						54	131	164	171	155		5	151
	166						42	111	183		131		16	118
	169						51	141	132					
Total	5226	226	159	106	127	1164	1691	4078	4396	4381	4593	135	580	4595
Average	174.2	9.0	7.5	5.8	8.4	43.1	56.3	135.9	146.5	156.4	158.3	6.4	20.0	158.4
% Normal	100	5	4	3	5	25	32	78	84	90	91	4	11	91

TABLE 10

Seedling Measurements of Mixed Radiation Diploid Dent Hybrid
Series 2b, Measured July 22, 1948

	Control	Lot Numbers of Samples												
		1	2	3	4	5	6	7	8	9	10	12	14	15
	193	9	10	99	10	160	161	236	193	208	317	15	44	279
	223	11	14	5	3	17	65	162	238	161	271	8	22	209
	222	13	7	9	7	61	154	189	200	285	280	10	15	238
	235	11	8	4	15	31	148	215	161	229	182	8	9	228
	234	13	16	7	17	22	35	195	214	194	200	9	6	290
	240	12	11	7	12	24	158	255	167	209	164	8	13	171
	269	11	9	3	13	90	51	219	160	286	212	4	14	241
	250	2	10	4	11	55	133	135	236	181	182	3	15	246
	233	8	12	9	16	16	49	201	124	270	220	9	23	209
	256	9	2	7	11	148	25	130	180	290	225	13	10	229
	230	10	5	9	7	112	90	171	229	250	221	6	15	200
	222	13	4	6	9	27	54	217	219	188	254	7	58	200
	262	6	7	7	12	44	69	167	208	239	224	11	5	157
	292	7	8	8	4	90	79	154	152	199	249	4	24	256
	283	2	10	7	8	36	15	175	233	174	166	5	10	259
	235	9	5	5		117	15	214	208	219	238	12	84	200
	245	12	11	5		85	150	159	165	158	234	3	17	225
	254	8	13			97	193	149	166	245	208	4	22	217
	291	7	3			134	172	195	156	191	188	9	12	266
	231	7	5			43	202	168	219	255	160	3	98	209
	255	12	6			79	110	148	210	167	177	6	7	167
	234	10				77	46	151	231	191	219		10	208
	268	16				70	144	168	174	219	281		61	227
	241					83	157	146	226	255	212		8	237
	270					93	19	176	193	249	227		29	229
	262					9	48	174	192	220	205		15	234
	255						15	150	166	274	207		14	225
	235						172	165	271	244	225		2	213
	237						158	139	264		155		3	150
	182						118	150	154				14	
									182					
Total	7339	318	176	111	155	1820	3005	5273	6091	6250	6303	157	674	6647
Average	244.6	13.2	8.3	6.5	10.3	70.0	100.1	175.7	196.4	223.2	217.3	7.4	22.4	221.5
% Normal	100	5	3	3	4	29	41	72	80	91	89	3	9	91

TABLE 11

Seedling Measurements of Mixed Radiation Lots of Tetraploid Dent
Series 2a, Measured July 17, 1948

	Control	Lot Numbers of Samples												
		1	2	3	4	5	6	7	8	9	10	12	14	15
	217	2	9	27	27	155	145	165	194	123	164	12	128	157
	214	7	20	5	22	131	139	134	147	160	151	10	121	175
	199	6	19	16	23	180	35	137	155	154	164	16	109	90
	162	11	13	18	12	152	129	142	130	159	168	7	128	158
	50	12	14	23	14	91	86	171	173	130	182	27	122	171
	25	6	20	23	18	146	152	168	149	135	144	15	104	177
	164	10	24	24	22	164	124	157	180	117	173	24	106	169
	177	10	14	26	5	152	132	161	179	189	167	17	117	147
	175	14	21	22	22	205	131	165	132	218	191	5	150	60
	222	16	22	18	18	134	142	183	153	106	172	16	117	125
	159	19	16	30	28	149	142	153	173	190	144	17	135	169
	170	7	19	25	10	170	126	166	154	171	159	12	137	22
	194	22	17	22	24	189	171	161	147	151	127	17	122	180
	181	4	17	22	23	146	169	156	154	154	81	11	138	189
	200	11	11	25	28	160	155	172	161	162	160	15	115	229
	191	10	12	19	20	105	111	157	177	140	160	20	134	145
	120	17	16	17	29	161	164	117	169	165	186	17	92	150
	162	19	22	19	20	130	134	176	167	142	133	12	77	141
	200	24	13	21	15	132	122	112	137	174	170	13	99	191
	173	7	17	19	21	158	139	116	138	149	130	9	119	182
	164	19	18	22	17	142	139	171	167	189	164		129	175
	156	12		19	18	134	152	156	154	179	163		123	149
	155	17		11	21	158	136	132	175	205	169		126	110
	140	22		17	20	150	143	119	166	156	170		125	181
	220			14	16	156	142	134	150	168	155		138	179
	191			20	22	61	115	145	140	165	158		128	145
	142				23	163	117	124	142	124	156		96	205
	133					144	135	144	114	144	155		38	147
	173					99	112	153	166	155			85	182
						156	128		104	164				
Total	4829	304	354	524	583	4373	3967	4347	4647	4738	4416	292	3358	4500
Average	166.5	12.6	16.8	20.1	19.9	145.7	132.2	149.8	154.9	157.9	157.7	14.6	115.7	115.1
% Normal	100	8	10	12	12	88	79	90	93	95	95	9	69	93

TABLE 12

Seedling Measurements of Mixed Radiation Lots of Tetraploid Dent
Series 2b, Measured July 22, 1948

	Control	Lot Numbers of Samples												
		1	2	3	4	5	6	7	8	9	10	12	14	15
	317	6	10	23	24	234	196	54	278	165	242	17	199	239
	235	8	22	15	22	270	230	246	193	220	204	10	175	262
	340	7	19	21	23	220	34	188	219	236	274	16	179	151
	326	14	14	18	11	242	214	181	193	258	278	6	175	252
	203	15	15	22	11	164	131	180	235	195	298	25	199	257
	52	9	18	24	17	228	241	262	229	181	202	17	168	290
	248	9	22	25	21	274	232	269	268	154	285	24	203	227
	303	15	15	22	22	259	198	273	272	293	267	12	185	206
	258	15	25	19	19	313	220	255	179	143	317	18	255	237
	309	19	21	28	27	160	235	262	245	323	284	15	183	201
	224	8	15	24	11	259	220	267	300	265	215	12	229	291
	208	22	19	24	21	257	201	233	217	251	264	10	214	290
	279	4	17	23	24	290	275	226	214	226	216	17	208	366
	285	10	17	24	29	200	259	224	231	209	130	8	230	218
	306	15	9	20	19	257	255	240	271	264	252	10	214	219
	298	18	9	17	20	172	205	273	263	224	246	15	212	222
	160	16	15	28	30	244	250	251	236	203	314	20	164	301
	269	23	20	20	23	169	226	202	226	248	171	17	135	302
	322	13	12	19	19	199	171	263	180	286	259	13	209	226
	284	14	14	21	18	231	197	154	218	226	239	14	178	189
	256	9	16	22	23	216	217	210	254	280	272	9	198	143
	197	17		10	17	171	241	250	247	277	274		188	289
	257	21		12	25	251	225	226	263	333	291		197	263
	197			13	18	249	193	225	223	274	268		55	202
	340			20	20	230	254	184	232	240	266		203	329
	251				6	234	182	190	200	239	270		178	195
	209				24	108	193	208	248	166	243		174	275
	260				24	186	246	205	177	244	196		37	56
	26					145	191	205	284	239			93	62
						232	190	215	154	211				
Total	7219	307	344	514	568	6664	6322	6621	6949	7073	7037	305	5237	6760
Average	248.9	13.3	16.3	20.5	20.2	222.1	210.7	220.7	231.6	235.7	251.3	14.5	180.5	233.1
% Normal	100	5	7	8	8	89	85	89	93	95	101	6	73	94

SCIENTIFIC DIRECTOR'S REPORT

OF ATOMIC WEAPON TESTS

AT ENIWETOK, 1948

ANNEX 12

BIOLOGICAL AND ANIMAL CONTAINER STUDIES

(SERVICE TEST NO. 7)

PART I

EXPOSURE OF BIOLOGICAL ASSAY MATERIAL TO IONIZING RADIATION DURING TESTS X-RAY AND YOKE

Task Group 7.6

Project Report

EXPOSURE OF BIOLOGICAL ASSAY MATERIAL TO IONIZING RADIATION DURING TESTS X-RAY AND YOKE

by

Captain R. H. Draeger, MC, USN

30 June 1948 Project 7.1-17/RS(BM)-12

CONTENTS

I. ABSTRACT

Selected biologic materials including seeds, grain insects, fungi and bacteria were exposed to ionizing radiation during tests X-ray and Yoke at distances from 250 to 1,000 yards. This irradiation was accomplished in exposure cans and packets as was done at Bikini. These materials were supplied by the U. S. Department of Agriculture, California Institute of Technology, Chemical Corps, at Camp Detrick, and Naval Medical Research Institute and have been returned to the supplying agency for study. The major portion of the biologic material following test X-ray was collected on X-ray day. The maximum temperature attained in the exposure cans and packets was 110°F. The collection of test Yoke material was delayed and incomplete due to inability to find all of the exposure cans.

II. OBJECTIVES

The objective of this project is to obtain information regarding the effects of atomic bomb ionizing radiation upon selected biologic materials. The selection of this material was largely based upon the Bikini experiments. However, the plan was not to duplicate but rather to supplement and extend this experience. In most instances the selection was based upon negative results previously obtained due to a low intensity of exposure. This was particularly true for bacteria, grain insects and certain biologicals. On the other had, Neurospora spores and cultures, corn and cotton seeds were included since marked phenotypic and genetic changes have already been obtained and it is now desired to conduct studies at higher intensities of radiation.

III. INTRODUCTION

A study of the effects of ionizing radiation upon lower forms of life and biologically active materials may be expected to cast light upon the nature of radiation effects and the problems of radiation illness. While the nature of these injuries is not well understood, it is likely that the basic changes are common to all living cells. A study of these changes in lower forms of life, in which rapid reproduction takes place, also offers the opportunity to observe genetic effects quickly.

The variation in susceptibility of living organisms to ionizing radiation is very great. Higher animals and man may be killed by 300 to 500 r while insects require around 10,000 r and many bacteria and spores will survive 100,000 r. Thus it will be seen that a comparatively much greater radiation dosage is required for studies involving lower forms of life.

The exposure of living material at comparatively close range to an atomic bomb explosion presents a difficult problem. Not only must the material be protected against blast and thermal radiation but must, in addition, be protected against harmful environmental temperature changes due to outdoor exposure.

The agencies which have cooperated in furnishing biologic test materials are listed below, together with the name of the official with whom contact was made and type of material furnished. In each instance, the cooperating agency has agreed to submit the results of these studies to be included as addenda to the report of this project.

(1) California Institute of Technology

Dr. G. W. Beadle, Chairman, Biology Division

(a) Neurospora spores and cultures

(b) Corn seed

(2) U. S. Department of Agriculture

Dr. P. V. Cardon, AEC Liaison Officer

(a) Seeds

(b) Grain insects

(c) Soils

(3) Chemical Corps, Camp Detrick

Dr. O. C. Woolpert, Technical Director

(a) Bacteria

(4) Naval Medical Research Institute

Captain R. H. Draeger, MC, U. S. Navy

(a) Biologicals

This report covers only the placement and recovery of the biologic materials exposed during Operation SANDSTONE. Reports concerning the irradiation effects will be issued later as addenda to this report.

IV. EXPERIMENTAL PROCEDURE

The following types of biologic test materials were selected for this project. A complete list of test materials and schedule of placement for tests X-ray and Yoke will be found in Appendix I.

(1) Neurospora Crassa

Spores, 34 sets

Cultures, 25 sets

(2) Seeds

Corn (M. T. Jenkins)	15 bags
Corn (E. G. Anderson)	4 bags
Cotton (M. S. Brown)	10 bags
Cotton (J. M. Weber)	10 bags

(3) Grain insects

Granary weevil	20 cans
Sawtooth grain beetle	20 cans
Red flour beetle	20 cans

(4) Soils

Type A	15 bags
Type B	15 bags
Type C	15 bags

(5) Bacteria	10 sets
(6) Biologicals	11 sets

This biologic material was collected at the Naval Medical Research Institute, Bethesda, Md. (except Neurospora which was obtained directly from California Institute of Technology) and shipped via government air to the U. S. Naval Shipyard, Long Beach, California and there placed aboard the U.S.S. Bairoko. The material was convoyed during air shipment and care taken not to subject it to extremes of temperature. Aboard ship it was stored in the air conditioned photographic laboratory at

70^{o} to $72^{o}F$. Following the tests, the biologic material was collected and again stored in the air conditioned laboratory after which it was shipped to the Naval Medical Research Institute via government air in the custody of an AEC Security Officer. The Neurospora spores and cultures were returned direct, via air, to Dr. G. W. Beadle at the California Institute of Technology.

Test station distances ranging from 250 to 1,000 yards, as follows, were chosen for the placement of the biologic materials for test X-ray and Yoke. Three departures from these selected distances were necessitated as noted below:

Station	Distance Yards
1	250
2	350
3	500
4	750
5	1,000

In test X-ray, Station 1 was increased to 330 yards in order to be outside the oiled area surrounding the bomb tower and Station 2 then increased to 400 yards. Also in test Yoke, Station 4 was reduced to 700 yards in order to avoid being in the water between Aomon and Biijiri islands.

The exposure of test materials at the first three stations of both test X-ray and Yoke was made in cylindrical steel containers approximately 6.5 inches in diameter and 28 inches long. The cylinders were fabricated from standard 6-inch galvanized iron pipe, one end being closed by a welded plate, the other by a pipe cap with four lugs welded on the side to facilitate removal (Fig. 1). These containers were attached to 4-inch galvanized iron stakes, 4.5 feet long, driven vertically about 2 feet into the ground. The exposure cylinders were supported within the Vee of the angle of the stake facing the direction of the blast by two clamps fabricated from 1/4- x 1-inch strap iron. Eight containers were used at each station or a total of 24 for each test.

The test materials were packed (see Figs. 2, 3, and 4) into thin galvanized iron cylindrical cans measuring 4 inches in diameter and 26 inches long in order to facilitate placement and removal from the steel exposure cylinders. In order to further protect the biologic material from shock and thermal effects, these inner cans were wrapped with 6 yards of flannel, 1 yard of canvas, and 1 yard of asbestos cloth and tied with glass cord. The completed packages were of such size that they fitted loosely into the steel exposure cylinders and could be easily removed.

The exposure of test materials at Stations 4 and 5 of test X-ray was made in four glass cloth covered packets, two at each station, supported by wooden structures as shown in Fig. 5. In order to prepare these packets measuring approximately 10 x 14 x 18 inches, the biologic material was assembled into a rectangular form, delicate items being packed into empty tin cans with flannel padding, and then wrapped with

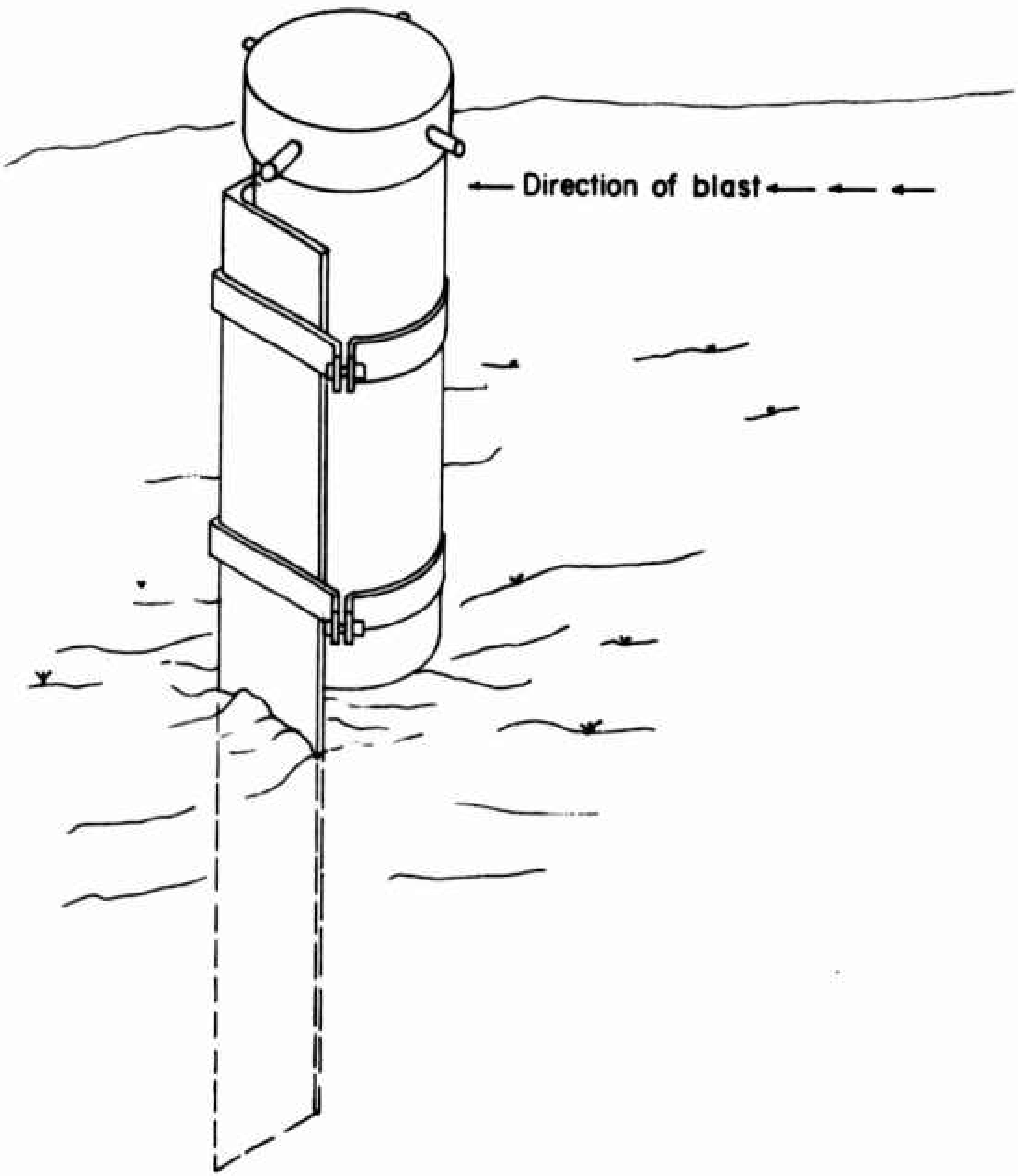

Fig. 1 Perspective View of Exposure Container for Biologic Material

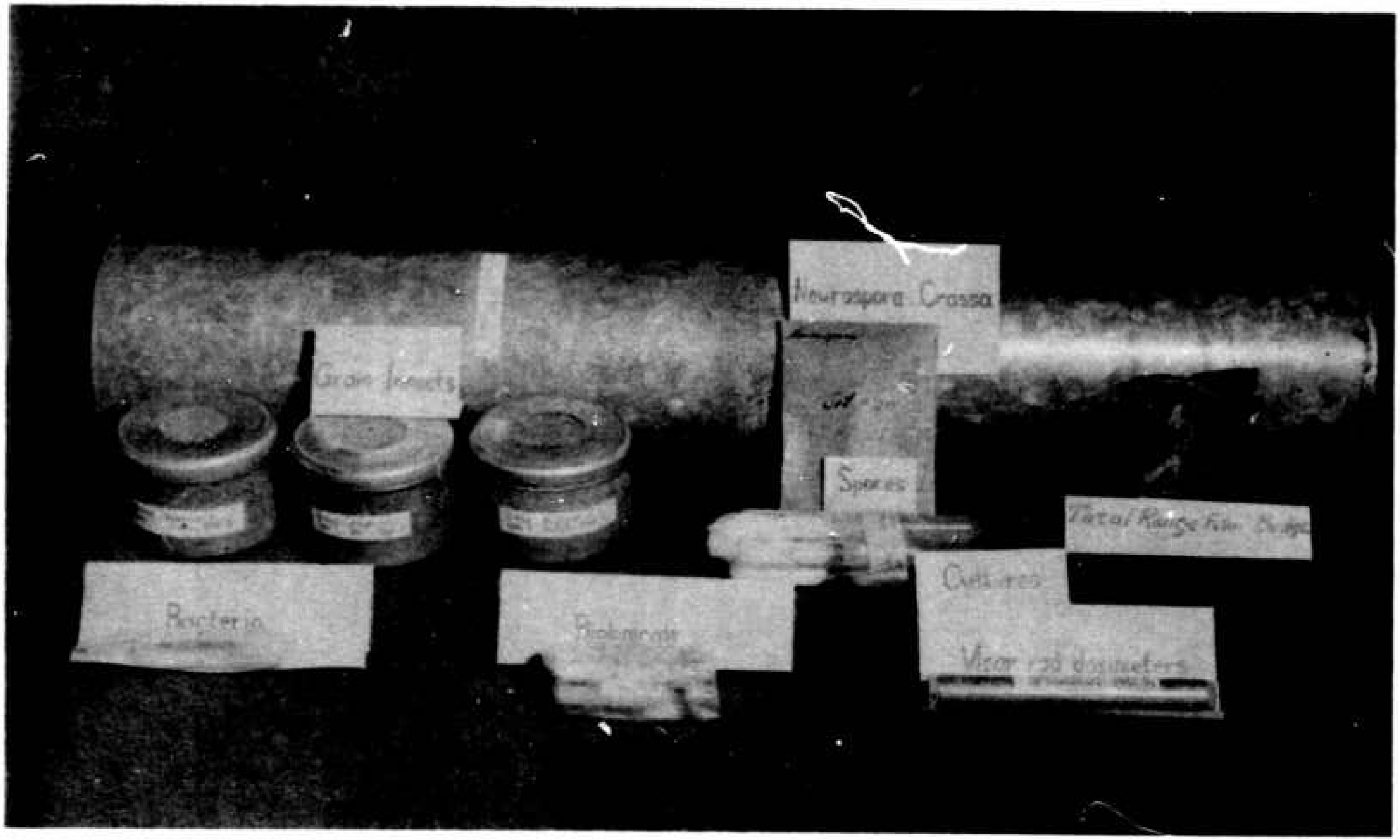

Fig. 2 Contents of an Inner Exposure Can

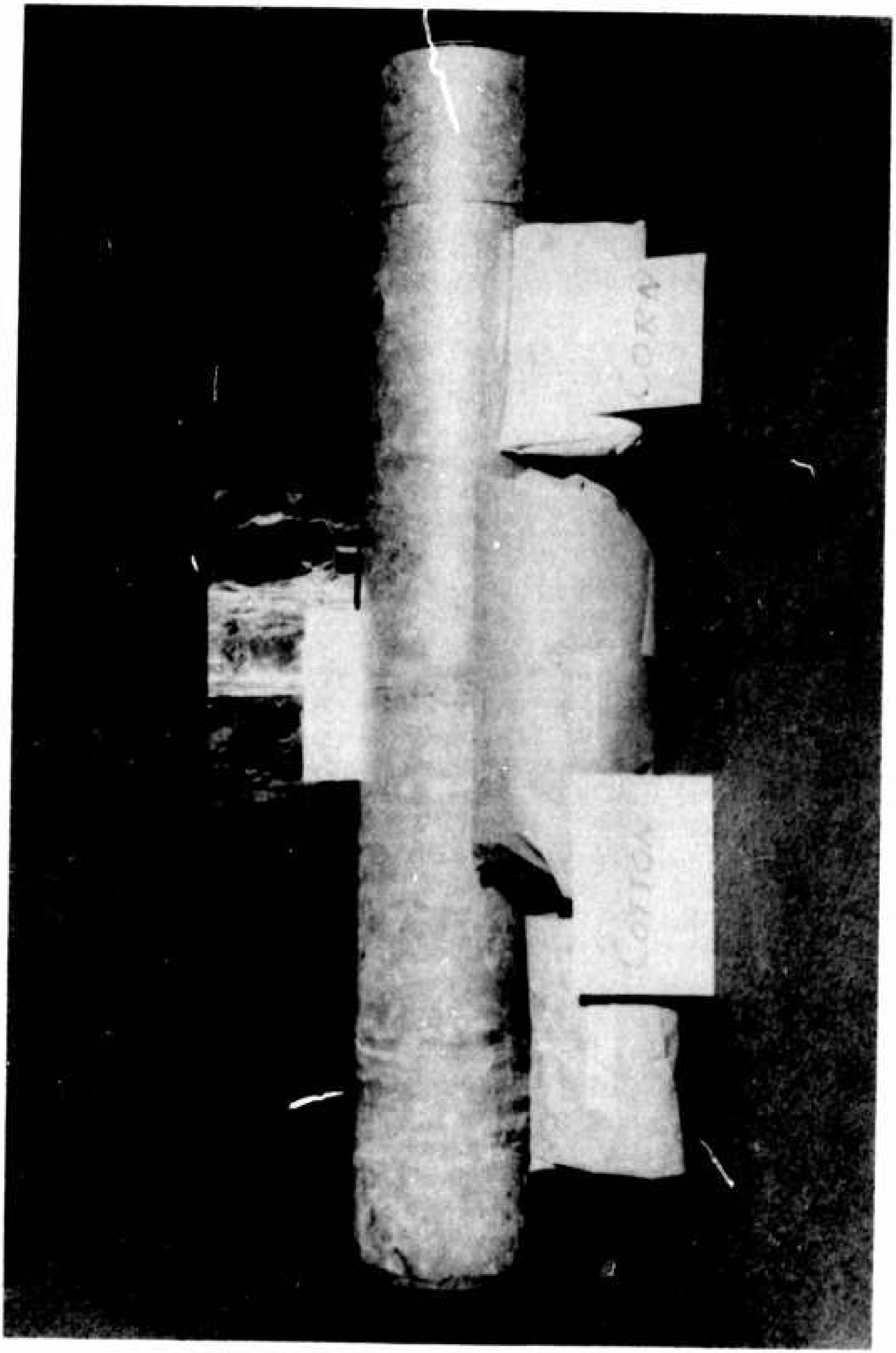

Fig. 3 Contents of an Inner Exposure Can Showing Cotton and Corn Seed

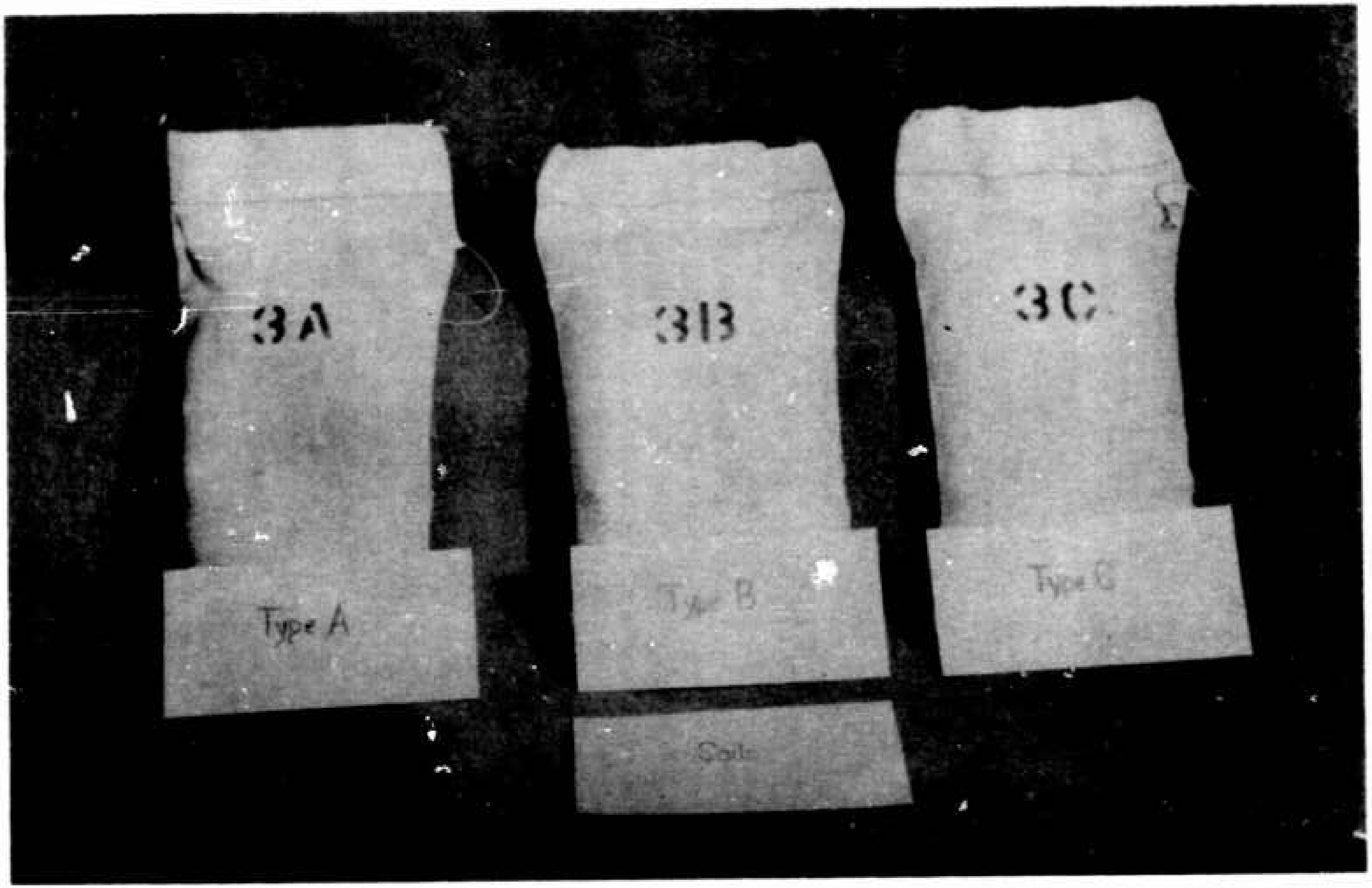

Fig. 4 Soil Samples to be Placed in Exposure Cans

Fig. 5 Biological Packets as Seen at Station 5 (1,000 yards) after Test X-ray

1 yard of canvas, 6 yards of flannel, 4 yards of canvas, 2 yards of asbestos cloth and finally 2 yards of glass cloth and secured with glass cord. The packets were also secured to the plywood faces of the wooden supports with glass cord. The exposure cans at Stations 1 to 3, test X-ray, were also covered on the outside with glass cloth secured by glass cord (Fig. 6). This was omitted in test Yoke for reasons which will become apparent.

The exposure of test material at Stations 4 and 5 of test Yoke was accomplished in four of the heavy galvanized iron cylinders, two at each station secured upon wooden structures very similar to those used in test X-ray to support the packets. This change in design was made in order to avoid the tedious wrapping of the packets and more adequately protect the test material from flying missiles. In this case the inner cans were not used but the material packed directly into the 6-inch iron cylinders. Since it was not expected that the containers were going to move appreciably at these comparatively distant stations, no special precautions were taken regarding shock protection.

The placement of the biologic test material for both Test X-ray and Yoke was effected on the day prior to each test in order to minimize exposure of the material to environmental conditions.

A study was made of the climatic thermal effects upon the internal temperature of the exposure containers. This was accomplished by measuring the maximum and minimum daily temperature attained for a period of several days in cans and packets under the following conditions.

Fig. 6 Exposure Containers at Station 1 - Test X-ray - Being Covered with Glass Cloth

(1) Galvanized can in sun

(2) Galvanized can in shade

(3) Aluminum painted can in sun

(4) Aluminum painted can in shade

(5) White painted can in sun

(6) White painted can in shade

(7) Glass cloth covered can in sun

(8) Glass cloth covered can in shade

(9) Galvanized can on ground following test X-ray

V RESULTS

The recovery of containers and packets was begun in the morning of X-ray day and completed the same day except for three cans one of which was found on the following day and the two remaining cans recovered on X-ray plus 2 days. As was not expected, the 4-inch angle iron stakes were bent over by the blast to an angle of about 60 degrees and the steel cylinders launched like rockets and scattered over a wide area. These distances are given in Table 1. The nearest container was found at 575 yards and the farthest at about 900 yards. The eight containers at Station 1 (330 yards) were thrown for an average distance of about 470 yards. The eight containers at Station 2 (400 yards) traveled for an average distance of about 275 yards while the eight containers at Station 3 (500 yards) traveled about 100 yards. It will be noted that the Station 1 cans traveled approximately five times as far as did Station 3 containers.

TABLE 1

Locations of Exposure Cans Following Test X-Ray

Distance in Yards from Original Position

Station 1 (330 yards)		Station 2 (400 yards)		Station 3 (500 yards)	
Can No.	Distance	Can No.	Distance	Can No.	Distance
1	523	9	No data	17	160
2	500	10	No data	18	No data
3	245	11	300	19	No data
4	455	12	375	20	100
5	485	13	No data	21	95
6	465	14	No data	22	93
7	565	15	212	23	128
8	524	16	215	24	93
Average	470.25		275.5		111.5

The fact that the exposure containers from several of the stations, particularly Stations 1 and 2, were displaced away from the bomb center (although not intended) was of considerable aid in the recovery of the exposure cans on X-ray day. As will be noted in Table 2 which lists the gamma radioactivity versus distance for several days, it would not have been possible to remain at Stations 1 and 2 for a sufficiently long time to remove the exposure cans on X-ray day had they remained upon their supports.

TABLE 2

Residual Gamma-ray Activity Following Test X-Ray

Distance in yards	Milliroentgens per hour			
	X-ray Day plus 6 hrs	X-ray plus one day	X-ray plus two days	X-ray plus three days
330		1,500	1,000	1,000
400		600	500	500
500	500	400	200	100
750	170	35	18	10
1,000		8	4	>4

It was hoped that the glass cloth covers placed on all 24 of the steel cylinders would withstand the thermal radiation and remain intact thus materially decreasing the subsequent absorption of solar radiation while awaiting recovery. This, however, did not occur, the glass cloth being completely stripped from the containers and the white paint also removed. The appearance of the cans as they were found upon the ground was quite similar to their original galvanized condition except slightly darker. Two of the containers suffered physical violence in the form of dents in their sides, however insufficient to prevent the removal of the inner containers. Several of the can covers were jammed requiring sledging before they could be removed.

The results of the temperature studies under the conditions listed are given in Table 3. The lowest maximum average temperature in the sun

TABLE 3

Temperature Measurements in Exposure Containers Under Various Conditions

		Degrees Fahrenheit								
		Mar 29	Mar 30	Mar 31	Apr 1	Apr 2	Apr 3	Apr 5	Apr 6	Average
Galvanized Can in Sun	Max	100	96	96	96	97	92	97	102	97
	Min	73	75	76	73	73	72	73	72	73
Galvanized Can in Shade	Max	93	93	91	90	90	91	93	91	91.5
	Min	75	74	77	74	74	72	73	76	74.4
Aluminum Painted Can in Sun	Max	93	92	94	94	93	96	95	97	94.2
	Min	73	75	75	73	73	72	72	72	73.1
Aluminum Painted Can in Shade	Max	87	86	87	88	86	87	86	87	86.8
	Min	73	75	76	73	73	71	73	76	73.8
White Painted Can in Sun	Max	91	90	91	91	90	92	91	93	91.1
	Min	73	75	75	73	72	73	72	72	73.1
White Painted Can in Shade	Max	88	86	87	88	87	87	87	87	87.1
	Min	73	76	77	75	74	75	77	73	75
Glass Cloth Covered Can in Sun	Max	-	91	93	94	92	94	93	95	93.1
	Min	-	75	76	73	74	72	74	72	73.7
Glass Cloth Covered Can in Shade	Max	88	88	85	88	86	88	88	87	87.2
	Min	73	73	76	73	74	75	78	77	74.9
			Apr 18		Apr 19		Apr 20			Average
Galvanized Can on Ground Following Test X-Ray	Max		110		107		107			108
	Min		76		77		77			76.6

was obtained in the white painted cans. The highest temperature we recorded in the galvanized cans on the ground.

The packets at Station 4 (750 yards) were torn from their supports which were displaced from their original position by a few yards. The surface of the glass cloth was tattered and torn due to pelting with missiles, but otherwise the packets were unharmed. Station 5 (1,000 yards) packets were recovered from their supports in their initial positions with their glass cloth coverings only slightly tattered.

The contents of the biological exposure cans suffered little damage in spite of having been thrown for a considerable distance. The contents of the packets were also in remarkably good condition. Except for clinical thermometers, practically all materials were recovered intact and returned to the appropriate agency for further study.

The recovery of exposure containers following test Yoke was far less successful than following test X-ray. On the morning of Yoke day an air observer reported that no exposure cans were seen lying about as following test X-ray. It was then decided to make a heliocopter flight over the area in an attempt to locate the exposure cans. This was accomplished on the afternoon of Yoke day and 10 exposure cans located in the shallow water between Aomon and Biijiri islands. An extensive search was also made of the shallow water between the islands and the reef in the direction of expected flight but no other exposure cans were located. On the following day (Yoke plus one day) the cans previously located by heliocopter flight were recovered from shallow water 4 to 8 feet deep between the islands by the author using a face mask and rubber boat. Subsequently, two other cans from Station 2 were located among palm tree debris on Aomon island at a distance of about 650 yards.

The location of the exposure cans recovered following test Yoke is given in Table 4. It will be noted that no cans were found from Station 1 and only two from Station 2. The ten cans found in the water between the islands were from Stations 3 and 4. These cans were all found within a radius of about 50 yards. No attempt was made to measure the positions in the water. Station 5 cans were found intact upon their supports.

TABLE 4

Location of Exposure Containers Recovered Following Test Yoke
Distance in yards from Original Position

Station 2 (350 yards)		Station 3 (500 yards)		Station 4 (700 yards)	
Can No.	Distance	Can No.	Distance	Can No.	Distance
34	300	41	about 300	49	about 75
35	325	42	about 300	50	about 100
		43	about 300		
		45	about 300		
		46	about 300		
		47	about 300		

The estimated radiation delivered to the biologic material as determined by film badges placed in each exposure container is given in Table 5.

TABLE 5

Estimated Gamma-ray Dosage at Test Stations

Test X-ray		Test Yoke	
Station	Radiation in r	Station	Radiation in r
1	over 140,000	1	film not recovered
2	over 140,000	2	over 140,000
3	55,000	3	over 122,000
4	10,000	4	7,500
5	2,500	5	2,500

VI. DISCUSSION

The exposure of moderately sensitive biologic material to atomic bomb ionizing radiation at close range presents a difficult problem. The exposure cans used were highly successful in test X-ray in spite of being launched like rockets and flying for hundreds of yards. The exposure containers were not expected to leave their supports. However, this occurrence had both advantages and disadvantages. The fact that Station 1 and 2 exposure cans traveled for about 470 and 275 yards respectively, placed them in a much more favorable position from the standpoint of recovery, ie., 500 to 675 yards from the bomb epicenter. The loss of exposure containers following test Yoke from Stations 1 and 2 was no doubt due to their being thrown over the reef. Had a greater distance been available on Aomon island, these cans would no doubt have been located as following test X-ray but with slightly greater damage to their contents.

There was considerable evidence of more violent blast effects upon the exposure containers following test Yoke. The angle iron stakes at Stations 1 and 2 were bent over to nearly 90 degrees while for test X-ray this angle was about 60 degrees. Stations 1 and 2 at 250 and 350 yards respectively were also closer than at test X-ray. The fate of the two exposure containers and supports at Station 4 (700 yards) is also evidence of greater violence. In spite of being ruggedly constructed and heavily staked down, these supports were carried away while the lighter supports used for test X-ray at 750 yards remained in place.

VII. SUMMARY AND CONCLUSION

Biologic materials were successfully exposed to atomic bomb ionizing radiation at distances greater than 330 yards from the epicenter and recovered on the afternoon of X-ray day.

The loss of exposure containers following test Yoke was probably caused by their being thrown over the reef. Had a greater distance been available, these containers would no doubt been successfully recovered.

The proper packing of delicate materials in an exposure container will enable them to withstand atomic bomb shock and blast effects up to 330 yards.

The temperature rise in the exposure containers to about 97°F on the day before the test, and afterward to about 110°F for six hours, is not expected to effect adversely any of the materials exposed.

APPENDIX 1

LIST OF BIOLOGIC ASSAY MATERIAL FOR TESTS X-RAY AND YOKE INCLUDING SCHEDULE OF PLACEMENT

I. Complete list of biologic assay material for Tests X-ray and Yoke.

A. Neurospora crassa

1. Spores, 34 sets

a. Sets 1 to 14 ----------------- 7 vials, each

b. Sets 14 to 34 ---------------- 4 vials, each

2. Cultures, 25 sets

a. Sets 1 to 10 ----------------- 6 culture tubes, each

b. Sets 11 to 25 ---------------- 8 culture tubes, each

B. Seeds

1. Corn (M. T. Jenkins) 15 bags, 6 varieties

a. L 289 x 1205

b. Strawberry popcorn

c. Perdue 51 (d22)

d. Tetraploid

e. F2 Waxy 289 x 205

f. Waxy 289

2. Corn (E. G. Anderson) 4 bags, variety not stated

3. Cotton (M. S. Brown) 10 bags, 23 varieties

a. Diploid species

(1) Gossypium anomalum

(2) Gossypium thurberi

(3) Gossypium davidsonii

(4) Gossypium arboreum var. Nanking

(5) Gossypium arboreum var. neglectum

(6) Gossypium arboreum var. Garo Hill

(7) Gossypium arboreum var. sanguineum

(8) Gossypium-multiple recessive stock

(9) Gossypium-multiple dominant stock

b. Hybrids

(10) Upland x Nanking - doubled z62

(11) Upland x Stocksii - doubled z288

(12) Upland x Garo Hill - doubled z77

(13) Z-114 hybrid octoploid

c. Marked Upland Stocks

(14) Multiple dominant S.L. 7-9

(15) Multiple dominant Carver 2-1

(16) Vy - OL

(17) Branched stamens, crinkled leaf

(18) Dark brown lint 5

(19) Green lint 4-1-1

(20) King Spot

d. Upland Varieties

(21) Acala Rogers

(22) Deltapine 15

(23) Stoneville 2B

4. Cotton (J. M. Weber) 10 bags of 3 lots

a. Lot No. 1

(1) CB101 Gossypium davidsonii ---------------50 seeds

(2) CB112 Gossypium thruberi -----------------50 seeds

(3) CB861 Gossypium harknessii ---------------35 seeds

(4) CB632 Gossypium sturtii -----------------100 seeds

(5) CB763 Gossypium stocksii ---------------- 45 seeds

(6) CBW170 Gossypium barbadense (Amsak) ----- 15 seeds

b. Lot No. 2

(1) CB867 Gossypium armourianum ------------- 40 seeds

(2) CB1223 Gossypium raimondii -------------- 15 seeds

(3) CB617 Gossypium aridum ------------------ 35 seeds

(4) CB167 Gossypium hirsutum (Acala) -------- 30 seeds

c. Lot No. 3

(1) CB1051 Gossypium anomalum --------------- 50 seeds

(2) CB700 Gossypium klotzschianum ----------- 50 seeds

(3) CBW15 Gossypium arboreum v. cernuum ----- 40 seeds

C. Grain insects, 3 species, 20 sets

1. Species

a. Granary weevil ------------------Sitophilus granarius

b. Sawtooth grain beetle -----------Oryzaephilus surinamensis

c. Red Flour beetle ----------------Tribolium castaneum

D. Bacteria, 6 species, 19 lots

1. Species

a. E. coli

b. Mycobacterium phlei

c. Serratia marcescens

d. Staphylococus aureus

e. Bacillus gabigii (BG)

f. Klebsiella pneumoniae

E. Biologicals, 9 substances, 11 lots

1. Substances

a. Papain

b. Albumin

c. Heparin

d. Ascorbic acid

e. Glutathione

f. Lipase

g. Diastase

h. Pepsin

i. Urease

F. Soils, 3 types, 15 bags of each

1. Types

a. Type A, 15 bags numbered 1 to 15

b. Type B, 15 bags numbered 16 to 30

c. Type C, 15 bags numbered 31 to 45

II. Schedule of placement of biologic assay material for Test X-ray.

As noted in the report, all exposure cans were found and their contents recovered with negligible loss following Test X-ray. A list of material recovered will be given in the individual reports by the several collaborating agencies.

A. Station 1 (330 yards)

1. Neurospora crassa

a. Spores, sets 1 to 4

b. Cultures, sets 1 and 2

2. Grain insects, sets 1 and 2

3. Soils, bags 1, 16 and 31

4. Bacteria, sets 1 and 2

5. Biologicals, set 1

B. Station 2 (400 yards)

1. Neurospora crassa

a. Spores, sets 5 to 8

b. Cultures, sets 3 and 4

2. Seeds

a. Corn (M. T. Jenkins) bags 1 and 2

b. Cotton (M. S. Brown) bag 1

c. Cotton (J. M. Weber) bag 1

3. Grain insects, sets 3 and 4

4. Soils, bags 2, 17 and 32

5. Bacteria, sets 3 and 4

6. Biologicals, set 2

C. Station 3 (500 yards)

1. Neurospora crassa

    a. Spores, sets 9 to 12

    b. Cultures, sets 5 and 6

2. Seeds

    a. Corn (M. T. Jenkins) bags 3 and 4

    b. Corn (E. G. Anderson) bag 1

    c. Cotton (M. S. Brown) bag 2

    d. Cotton (J. M. Weber) bag 2

3. Grain insects, sets 5 and 6

4. Soil, bags 3, 18 and 33

5. Bacteria, sets 5 and 6

6. Biologicals, set 3

D. Station 4 (750 yards)

1. Neurospora crassa

    a. Spores, sets 13 to 16

    b. Cultures, sets 7 and 8

2. Seeds

    a. Corn (M. T. Jenkins) bags 5 and 6

    b. Corn (E. G. Anderson) bag 2

    c. Cotton (J. M. Weber) bag 3

    d. Cotton (M. S. Brown) bag 3

3. Grain insects, sets 7 and 8

4. Soil, sets 4, 19 and 34

5. Bacteria, sets 7 and 8

6. Biologicals, set 4

E. Station 5 (1,000 yards)

1. Neurospora crassa

   a. Spores, sets 19 and 20

   b. Cultures, sets 9 and 10

2. Seeds

   a. Corn (M. T. Jenkins) bag 7 and 8

   b. Corn (E. G. Anderson) bag 3

   c. Cotton (M. S. Brown) bag 4

   d. Cotton (J. M. Weber) bag 4

3. Soil, sets 5, 20 and 35

III. Schedule for placement of biologic assay material for Test Yoke.

As stated in the report, only eight exposure cans were found following Test Yoke. The list of materials recovered will be given in the individual reports by the several collaborating agencies.

A. Station 1 (250 yards)

1. Neurospora crassa

   a. Spores, sets 21 and 22

   b. Cultures, sets 11 and 12

2. Seeds

   a. Corn (M. T. Jenkins) bag 11

   b. Cotton (M. S. Brown) bag 6

   c. Cotton (J. M. Weber) bag 6

3. Grain insects, sets 11 and 12

4. Soil, bags 6, 7, 21, 22, 36 and 37

5. Bacteria, sets 10 and 11

6. Biologicals, set 6

B. Station 2 (350 yards)

1. Neurospora crassa

a. Spores, sets 23 and 24

b. Cultures, sets 13 and 14

2. Seeds

a. Corn (M. T. Jenkins) bag 12

b. Cotton (M. S. Brown) bag 7

c. Cotton (J. M. Weber) bag 7

3. Grain insects, sets 13 and 14

4. Soil, bags 8, 9, 23, 24, 38 and 39

5. Bacteria, sets 12 and 13

6. Biologicals, set 7

C. Station 3 (500 yards)

1. Neurospora crassa

a. Spores, sets 25 and 26

b. Cultures, sets 15 and 16

2. Seeds

a. Corn (M. T. Jenkins) bag 13

b. Cotton (M. S. Brown) bag 8

c. Cotton (J. M. Weber) bag 8

3. Grain insects, sets 15 and 16

4. Soil, bags 10, 11, 25, 26, 40, and 41

5. Bacteria, sets 14 and 15

6. Biologicals, set 8

D. Station 4 (700 yards)

1. Neurospora crassa

a. Spores, sets 27 and 28

b. Cultures, sets 17 and 18

2. Seeds

a. Corn (M. T. Jenkins) bag 14

b. Cotton (M. S. Brown) bag 9

c. Cotton (J. M. Weber) bag 9

3. Grain insects, sets 17 and 18

4. Soil, bags 12, 13, 27, 28, 42 and 43

5. Bacteria, sets 16 and 17

6. Biologicals, set 9

E. Station 5 (1,000 yards)

1. Neurospora crassa

a. Spores, sets 29 and 30

b. Cultures, sets 19 and 20

2. Soil, bags 14, 15, 29, 30, 44 and 45

IV. Biologic materials retained on U.S.S. Bairoko as controls.

1. Neurospora crassa

a. Spores, sets 31 to 34

b. Cultures, sets 21 to 25

2. Seeds

a. Corn (M. T. Jenkins) bags 9, 10 and 15

b. Corn (E. G. Anderson) bag 4

c. Cotton (M. S. Brown) bags 5 and 10

d. Cotton (J. M. Weber) bags 5 and 10

3. Grain insects, sets 9, 10, 19 and 20

4. Bacteria, sets 9, 18 and 19

5. Biologicals, lots 5, 10 and 11

SCIENTIFIC DIRECTOR'S REPORT

OF ATOMIC WEAPONS TEST

AT ENIWETOK, 1948

Annex 12

BIOLOGICAL AND ANIMAL CONTAINER STUDIES

(SERVICE TEST NO. 7)

Part II

TEST OF CONTAINERS FOR EXPOSING ANIMALS

TO ATOMIC BOMB IONIZING RADIATION AT CLOSE RANGE

Task Group 7.6

Project Report

TEST OF CONTAINERS FOR EXPOSING ANIMALS TO ATOMIC BOMB IONIZING RADIATION AT CLOSE RANGE

by

Captain R. H. Draeger, MC, U.S. Navy

17 May 1949 Project 7.1-17/RS(BM)-14

CONTENTS

I. ABSTRACT

Four animal containers, two on rafts and two on land, were exposed to the blast effects of Test X-ray and Yoke during Operation SANDSTONE at distances from 350 to 1,500 yards in order to determine their suitability for use in irradiating large animals such as dogs or swine at close range. Studies of the maximum daily temperatures attained within the animal exposure chambers were made as well as observations of the effects of blast at the several distances. This data will be used as the basis for further laboratory experimentation at the Naval Medical Research Institute in order to establish satisfactory survival conditions for the animals during the exposure period.

II. OBJECTIVE

The objective of this project is to develop means for exposing large animals such as dogs or swine to high-intensity atomic bomb ionizing radiation in order to observe the early physiological, biochemical and pathological changes as part of a program for the study of acute radiation illness.

III. INTRODUCTION

With the advent of the atomic bomb the problem of radiation illness has become of great medical military importance. Although radiation illness following X-ray therapy has been known for many years, there has been little opportunity to observe the effects of high-intensity total-body irradiation. While thousands suffered acute radiation illness at Hiroshima and Nagasaki, little information regarding the histopathology of this condition in its early stages, is available. A large number of animals were exposed to atomic bomb ionizing radiation at Bikini during tests ABLE and BAKER, however no material was obtained during the early period following irradiation.

This project was undertaken as a first step toward the procurement of animal material needed to fill a gap in our knowledge of the histopathology and biochemistry of the early stages of radiation illness. The testing of the animal containers, as here reported, furnishes information needed for the design of equipment to protect the animals from adverse environmental effects during the exposure period.

IV. EXPERIMENTAL PROCEDURE

The four animal containers used in this project were constructed at the Terminal Island Naval Shipyard, Long Beach, California, following preliminary experiments at the Naval Medical Research Institute. Two of the containers were constructed for use on land and two were mounted on rafts to be anchored in shallow water off shore.

The following locations were used for the placement of the animal containers during atomic bomb tests X-ray and Yoke, Operation SANDSTONE.

TABLE 1

Location of Animal Containers During Tests X-ray and Yoke

Test X-ray	Test Yoke
No. 1 on raft at 1,000 yards	No. 1 on raft at 500 yards
No. 2 on raft at 1.500 yards	No. 2 on raft at 750 yards
No. 3 on land at 750 yards	No. 3 on land at 350 yards
No. 4 on land at 1,000 yards	No. 4 on land at 500 yards

The construction of the animal containers is shown in Fig. 1, which is a longitudinal section showing the manner in which animals are to be housed; although no animals were used in this project, the tanks only being tested. The container consists essentially of a 1/2-inch-thick steel cylinder 28 inches in diameter and 6 feet long having conical ends 2 and 3. Conical end 3 is flanged as shown at 4 and retained by bolts 5 by which means it may be removed. The patch plate 6, Figs. 2 and 4, was added to facilitate access to the interior of the containers on rafts. The intended position of the animals 7 and 8 is shown within their respective cages 9 and 10.

Two of the tanks were mounted on rafts for use off shore as shown in Fig. 2 and two were fitted with a device, 11, to prevent rolling, were located on land as shown in Fig. 3. Figure 4 shows the patch plate cover removed for the purpose of making temperature measurements. All animal containers were painted white in order to reduce solar heating effects.

Temperature measurements were made within the containers by means of maximum-minimum thermometers in several positions in order to determine the diurnal temperature variations.

V. RESULTS

The results of the thermal measurements in the animal containers are given in the following tables.

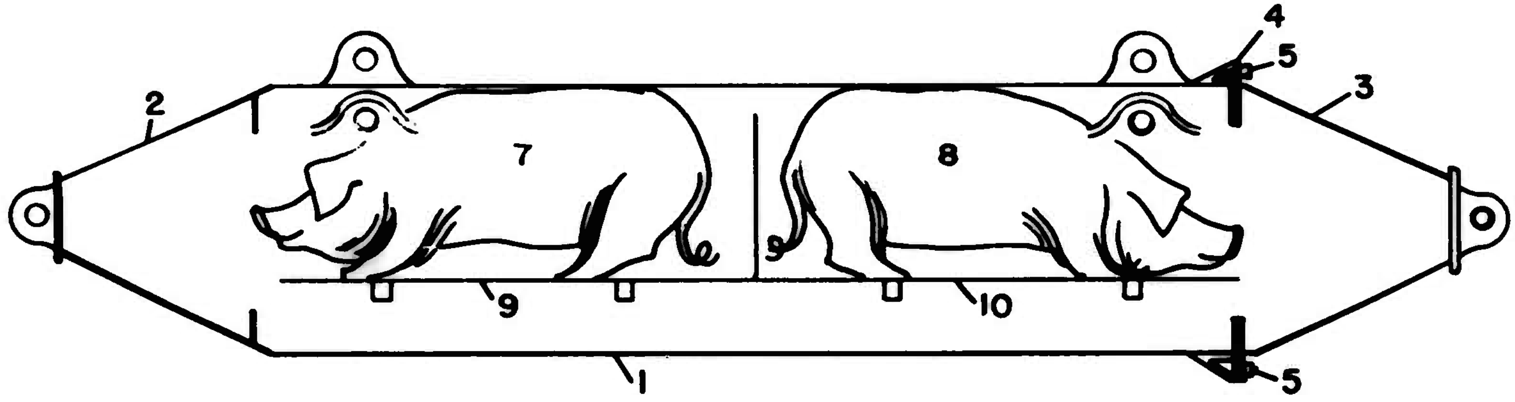

Fig. 1 Animal Container, Longitudinal Section

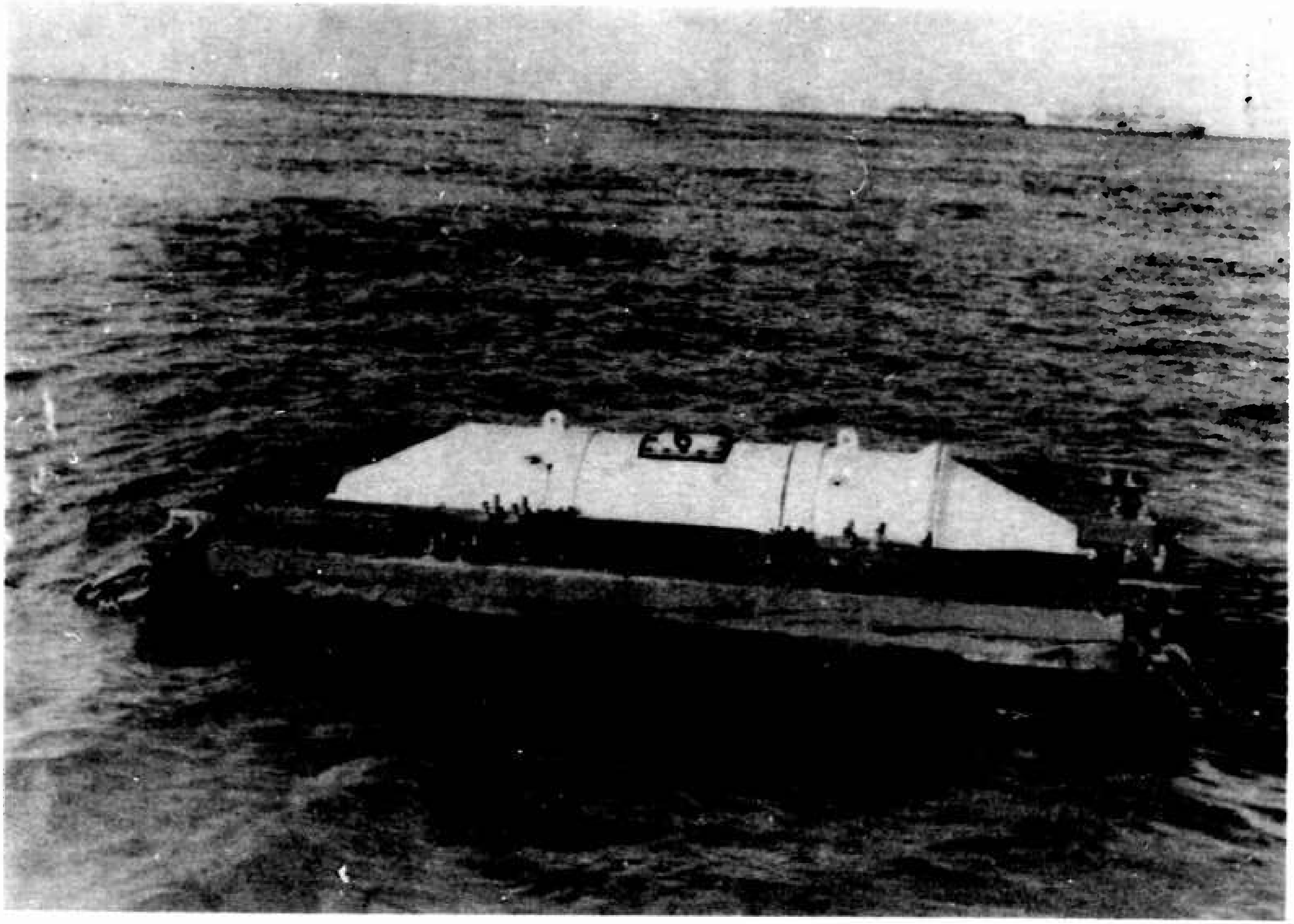

Fig. 2 Animal Container on Raft

Fig. 3   Animal Container for Use on Land

Fig. 4 Recording Temperature of Animal Container

TABLE 2

Maximum and Minimum Temperatures Recorded in Animal Containers on Rafts in Degrees Fahrenheit

Date		Container No. 1		Container No. 2	
		Max.	Min.	Max.	Min.
9 April	Top	92	75	95	74
	Bottom	85	77	87	76
10 April	Top	94	77	95	76
	Bottom	85	78	87	78
11 April	Top	91	77	93	77
	Bottom	85	78	88	78
12 April	Top	90	75	92	77
	Bottom	86	77	87	77
13 April	Top	85	76	86	76
	Bottom	82	78	82	77
26 April	Top			86	76
	Bottom			87	78
27 April	Top			89	78
	Bottom			85	78
28 April	Top			86	77
	Bottom			83	78
29 April	Top			87	77
	Bottom			85	78

The temperature of the surface sea water measured daily at about 1000 ranged from 79° to 80° F.

TABLE 3

Maximum and Minimum Temperatures Recorded in Animal Containers on Land in Degrees Fahrenheit

Date	Container No. 3		Container No. 4	
	Max.	Min.	Max.	Min.
26 April	96	77	97	77
27 April	98	76	98	77
28 April	100	78	99	78
29 April	100	77	100	78

The maximum daily temperatures were recorded at about 1400 while the minimum temperatures were attained at about 0600 during the night.

There was no blast damage to any of the animal containers during Test X-ray. As far as could be ascertained the containers on rafts were not moved. Container No. 3 at 750 yards on land was moved radially about 3 feet while container No. 4 at 1,000 yards was not moved.

During Test Yoke container No. 1 on raft at 500 yards was overturned and one of the outer longitudinal wooden members cracked. No damage was done to the metal container. Raft No. 2 at 750 yards appeared to be in its original position. Containers No. 3 and 4 at 350 and 500 yards were displaced about 20 and 8 feet respectively. There was no blast damage to the containers.

The amount of displacement of the containers on land was definitely established by an iron stake driven at one end of each tank which served as a reference point.

VI. DISCUSSION

It will be noted that the experimental design of this project did not include the use of animals or the provision of survival conditions within the animal containers. The results indicate what conditions must be overcome rather than how this is to be done.

The daily maximum rise in temperature of the containers on rafts was to about 95°F. Similar measurements in containers on land showed a rise to about 100°F. A certain amount of cooling occured due to contact of the containers on rafts with sea water. The lower portion of these containers remained at about 5 degrees lower temperature. At night, however, the reverse condition prevailed, the upper part of the container cooling to about 2 degrees below the water temperature of 80°F. All measurements refer to air temperatures within the containers not to the surface temperature of the container.

If it is desired to expose animals at a range of 750 yards or more it is obvious from these tests that the container may be of much lighter construction.

VII. CONCLUSIONS

Animal containers of the type tested are suitable for the exposure of large animals to ionizing radiation beyond 750 yards from the standpoint of blast damage. Lighter construction may be used.

The daily temperature rise within the exposure containers both on rafts and on land was beyond the survival limits. Ventilation and cooling are definitely required.

For the tests X-ray and Yoke which were at comparatively low altitude the recovery of animals at less than 1,000 yards would probably have been more readily accomplished on the rafts than on land due to radioactivity. With increased altitude of the detonation this advantage would decrease.