OAK RIDGE NATIONAL LABORATORY
operated by
UNION CARBIDE CORPORATION
NUCLEAR DIVISION
for the
U.S. ATOMIC ENERGY COMMISSION

ORNL-TM-2235

BIOLOGY DIVISION

NEUROSPORA EXPERIMENT P-1037

QUARTERLY PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

July 1 - September 30, 1967

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BIOLOGY DIVISION

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TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

July 1 — September 30, 1967

NOVEMBER 1968

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee
operated by
UNION CARBIDE CORPORATION
for the
U.S. ATOMIC ENERGY COMMISSION

QUARTERLY PROGRESS REPORT
TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Title of Project:

Mutagenic Effectiveness of Known Doses of Gamma Irradiation in Combination with Zero Gravity on Neurospora

For the Period:

July 1 – September 30, 1967

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I. INTRODUCTION

Previous quarterly reports in this series are ORNL-TM 1734, ORNL-TM 1959, ORNL-TM 2189, and ORNL-TM 2190. The first of these includes results of early experiments (1) to determine the genetic effects of ^{85}Sr irradiation by estimating the frequencies of recessive lethal mutations at specific loci with a two-component heterokaryon of Neurospora crassa, (2) to determine the effects of anoxia, and (3) to evaluate the prototype and flight hardware in a series of biocompatibility tests performed in connection with the qualification and acceptance tests of the hardware in Philadelphia. ORNL-TM 1959 is concerned primarily with the tests, launch simulations, and gantry exercises at Cape Kennedy prior to the Biosatellite A flight as well as the preparations for flight of Biosatellite A. ORNL-TM 2189 describes the Neurospora experiment in the Biosatellite A flight and the analysis of the ground-control samples from this flight attempt. Some of the IBM data-collection sheets and data print-outs are also described and illustrated in this report. ORNL-TM-2190 describes the Con-Rad passive dosimetry system used in our early work and our attempts to identify some of the sources of variation in the data obtained with this system.

The present report discusses the following activities during the period July 1–September 30, 1967: (1) the radiation dosimetry test at Cape Kennedy in July, (2) the 201 Gantry Exercise at Cape Kennedy, (3) the 301 Gantry Exercise at Cape Kennedy, and (4) preparation for and completion of the successful Biosatellite B flight.

II. RADIATION-DOSIMETRY TEST AT CAPE KENNEDY

On July 17-20, in the Hangar S Annex at Cape Kennedy, dosimeters of three different types were inserted into the Neurospora experiment hardware and the individual packages were placed in the fore and aft sections of the 201 spacecraft. Attached to each package were tubes of lithium fluoride powder supplied by Ames Research Center. Three 12-hr exposures to gamma rays from an 85-strontium source were completed during the 4-day period. Representatives conducting the Tribolium, Habrobracon, and Tradescantia experiments carried out similar tests with their hardware, so that Ames Research Center personnel could estimate the radiation exposures to the biological samples in each of the radiation experiments.

The purposes of the experiment were: (1) to estimate in advance the radiation exposures that would be received by the biological samples in each of the radiation experiments in the Biosatellite II flight; (2) to provide the basis for an independent estimate of the exposures to be received in the Biosatellite II flight by extrapolating from thermoluminescence readings of the LiF powder to be irradiated, during flight, in positions on the exterior surface of the individual assemblies; (3) to estimate that portion of the ionizing radiation exposures resulting from low-energy scattered radiation; (4) to permit the experimenters to obtain experience with the thermoluminescent dosimetry systems, and to test the procedures for comparing the results obtained with the various systems; and (5) to obtain a calibration curve with ^{85}Sr gamma radiation (with individually calibrated dosimeters) and thus provide a basis for comparing data obtained by different experimenters with different dosimetric systems.

Because the description of some of the developmental work on the dosimetry system has not yet been completed, the detailed results from the Cape Kennedy test will be described in a subsequent report. In brief, it is possible to obtain separate estimates of the roentgen exposures from three different types of dosimeters (i.e.,

E.G. & G. LiF dosimeters, E.G. & G. CaF_2:Mn dosimeters, and Con-Rad LiF teflon-disk dosimeters) which are in good agreement. Furthermore, it was shown during this exercise that scattered radiation with energy of less than about 100 kev represented an average of 6.3% of the total exposure to the Neurospora samples.

III. 201 GANTRY EXERCISE

The 201 Gantry Exercise was performed in two separate steps. The first portion of the test required the experiment teams to prepare the biological materials and to coordinate their work with other groups on schedule. The biological materials were taken to the clean room in their individual assemblies and loaded into the fore and aft payloads on schedule. The payloads were placed into their carrying cases and vehicles, and these were moved as far as the door of Hangar S. After this, the payloads were disassembled and the biological materials were returned to the experimental teams in their individual assemblies. This first portion of the test was carried out with a simulated launch count-down time on August 3, 1967, and provided much-needed practice for both the new module-preparation team at ORNL and the assembly-coupling team at Cape Kennedy. At a later time, the second half of the 201 Gantry Exercise was completed, without biological materials; i.e., the assemblies and payloads were transported to the gantry, loaded into the spacecraft, and the count-down and check-outs were continued until just before launch.

Because the 301 Gantry Exercise was scheduled immediately prior to flight, with launch dates of August 28 for the 301 test and September 7 for flight, the Neurospora samples from the 201 Gantry Exercise were used in a forward-mutation exercise at ORNL. In this way, new ORNL personnel could become acquainted with the entire assay procedure and could acquire experience in new responsibilities in the assay procedure. Three modules from the 201 test were selected and irradiated

with 250 kvp X rays (30 mA, 3 mm aluminum extrinsic filtration) so that the first filters received an exposure rate of approximately 500 R/min for 3, 6, or 12 min, respectively. An additional module was retained as an unirradiated control. The conidia from filter Nos. 1 and 2 in the control- and 1500 R-modules, and those from filter Nos. 1, 2, and 3 from the 3000 R- and 6000 R-modules, were suspended and used in platings and jug inoculations in the customary manner. The data from these 201 Gantry Exercise conidia are presented in Table 1, and the average mutation frequencies for each filter sample are plotted in Fig. 1. In this figure, for comparison, some data are included from a previous experiment with conidia on filters which received total exposures of 5000, 10,000, and 20,000 R at a rate of 1000 R/min. The agreement with the forward-mutation frequencies expected by extrapolation of the dose-response curves is excellent, indicating that the procedures had been carried out satisfactorily.

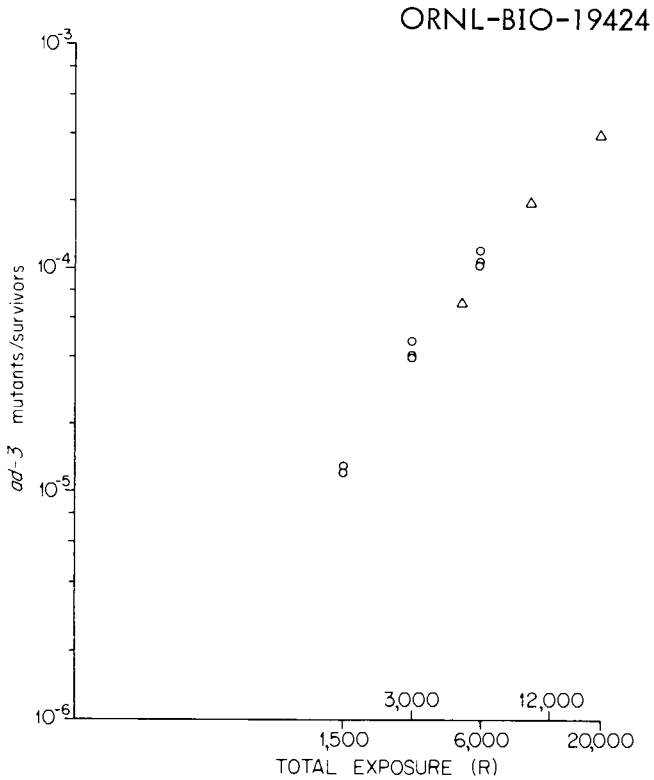

Fig. 1. Treatment Means for the Forward-Mutation Frequencies in Conidia Subjected to Storage in the 201 Gantry Exercise and then to 250 kvp X Rays at 500 R/min

O = X-ray data at 500 R/min from 201 Gantry Exercise; Δ = X-ray data at

Table 1

Data from a forward-mutation experiment inoculated with conidia from the 201 Gantry Exercise of August 1967

Arbitrary Treatment No.	Approximate X-Ray Exposure (R)	Filter No.	Plating Data: Heterokaryotic Survival: Proportion of All Conidia	Plating Data: Heterokaryotic Survival: Percent of Zero Dose (0.2025)	Jug Data: Jug No.	Jug Data: Heterokaryotic Survival: Proportion of All Conidia	Jug Data: Heterokaryotic Survival: Percent of Zero Dose (0.1472)	Jug Data: Purple Colonies	Jug Data: Estimated Background Colonies	Jug Data: Forward Mutation Frequency ($\times 10^6$)
1	0	1	0.1934	95.5	1	0.1424	96.7	1	694,720	1.4
					7	0.1522	103.4	0	742,560	0
					37	0.1348	91.6	0	657,760	0
2	0	2	0.2116	104.5	2	0.1950	132.5	1	838,662	1.2
					38	0.1370	93.1	0	589,132	0
					39	0.1391	94.5	0	598,005	0
					40	0.1302	88.5	0	559,861	0
3	1500	1	0.1417	70.0	3	0.1390	94.4	4	586,042	6.8
					12	0.1209	82.1	7	509,763	13.7
					21	0.1114	75.7	7	469,960	14.9
					34	0.1369	93.0	8	577,231	13.9
4	1500	2	0.1149	56.7	4	0.1365	92.7	8	647,473	12.4
					25	0.1289	87.6	8	611,325	13.1
					31	0.1259	85.5	8	591,863	13.4
5	3000	1	0.1151	56.8	5	0.0817	55.5	27	391,959	68.9
					14	0.1256	85.3	29	602,950	48.1
					16	0.1079	73.3	22	517,722	42.5
					23	0.1136	77.2	20	545,140	36.7
6	3000	2	0.1169	57.7	6	0.1081	73.4	20	522,226	38.3
					15	0.0941	63.9	15	454,837	33.0
					24	0.0928	63.0	18	448,339	40.1
					33	0.1198	81.4	29	578,867	50.1
7	3000	3	0.1391	68.7	8	0.1075	73.0	18	469,279	38.4
					17	0.1013	68.8	23	442,309	52.0
					26	0.0753	51.2	16	328,640	48.7
					35	0.1075	73.0	11	469,665	23.4
8	6000	1	0.1162	57.4	9	0.0955	64.9	42	456,625	92.0
					18	0.1062	72.1	64	507,901	126.0
					27	0.0974	66.2	60	465,920	128.8
					30	0.1014	68.9	67	485,109	138.1
9	6000	2	0.1085	53.6	10	0.0946	64.3	51	422,433	120.7
					19	0.1036	70.4	45	462,697	97.3
					22	0.1011	68.7	48	451,827	106.2
					28	0.0900	61.1	40	401,920	99.5
10	6000	3	0.1039	51.3	11	0.1035	70.3	54	446,038	121.1
					20	0.0996	67.7	49	429,113	114.2
					29	0.0972	66.0	46	418,633	109.9
					32	0.1192	81.0	44	513,431	85.7
Means:										
1	0	1	0.1934	95.5	1, 7, 37	0.1431	97.2	1	2,095,040	0.5
2	0	2	0.2116	104.5	2, 38, 39, 40	0.1503	102.1	1	2,585,787	0.4
3	1500	1	0.1417	70.0	3, 12, 21, 34	0.1271	86.3	26	2,142,996	12.1
4	1500	2	0.1149	56.7	4, 25, 31	0.1304	88.6	24	1,850,661	13.0
5	3000	1	0.1151	56.8	5, 14, 16, 23	0.1072	72.8	98	2,057,771	47.6
6	3000	2	0.1169	57.7	6, 15, 24, 33	0.1037	70.4	82	2,004,269	40.9
7	3000	3	0.1391	68.7	8, 17, 26, 35	0.0947	64.3	68	1,709,893	39.8
8	6000	1	0.1162	57.4	9, 18, 27, 30	0.1001	68.0	233	1,915,555	121.6
9	6000	2	0.1085	53.6	10, 19, 22, 28	0.0973	66.1	184	1,738,877	105.8
10	6000	3	0.1039	51.3	11, 20, 29, 32	0.1049	71.3	193	1,807,215	106.8
1 & 2	0		0.2025	100.0		0.1472	(100.0)	2	4,680,700	0.4
3 & 4	1500		0.1283	63.4		0.1299	88.2	50	3,993,657	12.5
5, 6, & 7	3000		0.1237	61.1		0.1029	69.9	248	5,771,933	43.0
8, 9, & 10	6000		0.1095	54.1		0.1007	68.4	610	5,461,647	111.7

IV. 301 GANTRY EXERCISE

For the 301 Gantry Exercise, biological samples were loaded into 15 modules at ORNL on August 24. These modules were transported at ice-water temperature to Cape Kennedy, where they were held in reserve in case of subsequent emergency. The 15 additional modules actually used in the test were loaded on August 26 and were shipped to Cape Kennedy on August 27. A copy of the Requisition and Invoice/ Shipping Document (DD Form 1149) for these modules is included as Table 2. The third set of 15 modules, to be used in case of a test delay of about 2 days, was prepared on August 28 and transported to Cape Kennedy on August 29.

The purpose of the 301 Gantry Exercise was to provide a final check of the actual flight hardware, and to assure that the experiment teams (some of which included relatively inexperienced personnel) could participate effectively in the count-down and flight periods. For the Neurospora experiment, this exercise was completely successful. The count-down for the simulated launch on August 28 was completed on schedule, and the work schedules for coverage of the Hangar S laboratory during the simulated flight period of 3 days by each experimental group were firmly established. It was also possible, at this time, to finalize the schedules of activities for all the experimental groups in case of a possible emergency or delay that might require the preparation of new biological material.

The biological samples from the 301 Gantry Exercise were not analyzed because such analysis would have conflicted with preparations for the actual flight. The dosimeters were saved and used later to estimate the radiation exposures to each of the filters during the 301 Gantry Exercise.

Table 2

SHIPPING CONTAINER TALLY

REQUISITION AND INVOICE/SHIPPING DOCUMENT

SHEET NO. 1 / NO. OF SHEETS	5. REQUISITION DATE
6. REQUISITION NO.	
1. FROM: NAS-2-1900 ~~Oak Ridge National Laboratory, Biology Div., Oak Ridge, Tenn.~~	7. DATE MATERIEL REQUIRED
8. PRIORITY	
2. TO: Transporation Officer, Patrick AFB, Flordia General Electric Co. ETR, Hanger "S" Cape Kennedy, Flordia Attn: J. R. Krepps M/F NAS 2-1900	9. AUTHORITY OR PURPOSE: J. Glancey/Hemium Agreement
10. SIGNATURE	11a. VOUCHER NUMBER AND DATE: CS67-19-161
3. SHIP TO -MARK FOR: Same as above	12. DATE SHIPPED: 8/27/67
b. VOUCHER NUMBER AND DATE: 8/5/67	13. MODE OF SHIPMENT: Hand carried
14. BILL OF LADING NUMBER	15. AIR MOVEMENT DESIGNATOR OR PORT REFERENCE NUMBER

4. ACCOUNTING AND FUNDING DATA

80X0108 (64) 2510 - R- 2100

ITEM NO. a	FEDERAL STOCK NUMBER, DESCRIPTION, AND CODING OF MATERIEL AND/OR SERVICES b	UNIT OF ISSUE c	QUANTITY REQUESTED d	SUPPLY ACTION e	TYPE CONTAINER f	CONTAINER NOS. g	UNIT PRICE h	TOTAL COST i
1	47D168798G1, Neurospora Module, S/N 15A	ea.	1					
2	" " " " 16	"	1					
3	" " " " 16A	"	1					
4	" " " " 17	"	1					
5	" " " " 17A	"	1					
6	" " " " 18	"	1					
7	" " " " XVII	"	1					
8	" " " " XVIII	"	1					
9	" " " " XXI	"	1					
10	" " " " XXII	"	1					
11	" " " " XXIII	"	1					
12	" " " " XXIV	"	1					
13	" " " " XXV	"	1					
14	" " " " V	"	1					
15	" " " " XVI	"	1					

16. TRANSPORTATION VIA MATS OR MSTS CHARGEABLE TO

17. SPECIAL HANDLING

RECAPITULATION OF SHIPMENT

18. ISSUED BY	TOTAL CONTAINERS	TYPE CONTAINER	DESCRIPTION	TOTAL WEIGHT	TOTAL CUBE
CHECKED BY					
PACKED BY					
		←	TOTAL	→	

RECEIPT

19.	DATE	BY	
CONTAINERS RECEIVED EXCEPT AS NOTED			SHEET TOTAL
QUANTITIES RECEIVED EXCEPT AS NOTED			GRAND TOTAL
POSTED			20. RECEIVER'S VOUCHER NO.

DD FORM 1 MAR 59 1149 REPLACES EDITION OF WHICH MAY BE USED

V. FLIGHT OF BIOSATELLITE B

A. Personnel and Responsibilities

Specific responsibilities for flight or launch simulations, as well as the assignment of these responsibilities to particular people for the Biosatellite A flight, were described previously (ORNL-TM 1959). During the period between the launch dates of Biosatellite A and Biosatellite B, it became necessary to alter these assignments because of replacements or other changes in status as shown in Table 3. The major new assignment in regard to preparation for flight was the inclusion of Mrs. Miller as the third person in the assembly team; her continuing responsibility for dosimetry during the past year made her the logical choice for keeping track of the three different types of passive dosimeters which were now being used to measure the radiation exposure in each sample position. Another change was the distribution of duties in the post-flight plating and forward-mutation experiments among a larger number of personnel. This distribution, as well as the rehearsal of the responsibilities in the 201 and 301 Gantry Exercises and the forward-mutation exercise following the 201 Gantry Exercise, was an important factor in permitting the rapid completion of the plating, jug inoculation, and jug harvesting for this 180-jug experiment. Table 4 indicates the traveling and work schedules for the pre-flight and flight-readiness periods, while Table 5 presents the assignments for responsibilities during the post-flight period.

Table 3. Changes in responsibilities of personnel for the Neurospora experiment in the Biosatellite B flight

Responsibilities	Personnel	
	Biosatellite A	Biosatellite B
Principal investigator; experimenters' representative	F. J. de Serres	F. J. de Serres
Coinvestigator; Samoan contingency and communications at Hickam Field	B. B. Webber	B. B. Webber
Receipt of assemblies in recovery area; assist in preparation for Samoan contingency	I. C. Miller	A. P. Teasley
Coupling of assemblies and coverage of Hangar S laboratory	E. C. Gourley D. S. Carroll	E. C. Gourley D. S. Carroll
ORNL module preparation team	M. C. Gibson A. P. Teasley	M. T. Sheppard P. E. Harris I. C. Miller
Transport team No. 1	J. S. Wassom D. W. Ramey	J. S. Wassom S. H. Lyon
Transport team No. 2	L. Oggs L. B. Ralston	L. B. Ralston W. K. Barnett
Transport team No. 3	M. T. Sheppard P. E. Harris	D. W. Ramey M. D. Shelby
Alternates for transport	W. P. Henry	P. N. Gambill

Table 4. Schedules for pre-flight and flight-readiness periods

Revised 8/17/67	August									September																			
	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Event	301 Gantry T-O									Flight readiness T-O X X X																			
									Genetics Society																				
F. J. de Serres	Cape Kennedy								Stanford			Cape Kennedy					Honolulu (?)				Oak Ridge (?)								
B. B. Webber									Stanford				Honolulu								Oak Ridge (?)								
A. P. Teasley															Honolulu						Oak Ridge (?)								
E. C. Gourley									Cape Kennedy												Oak Ridge (?)								
D. S. Carroll									Cape Kennedy												Oak Ridge (?)								
J. S. Wassom (T1)			Cape Kennedy										Cape Kennedy							Cape (?)									
S. H. Lyon (T1)			Cape Kennedy										Cape Kennedy																
L. B. Ralston (T2)					Cape Kennedy										Cape Kennedy						Cape (?)								
W. K. Barnett (T2)					Cape Kennedy										Cape Kennedy						Cape (?)								
D. W. Ramey (T3)							Cape Kennedy									Cape Kennedy						Cape (?)							
M. D. Shelby (T3)							Cape Kennedy									Cape Kennedy						Cape (?)							
P. N. Gambill																			Cape (?)										
Module Preparation			*		*		*					*		*		*		(*)		(*)		(*)							
M. T. Sheppard P. E. Harris I. C. Miller																													

Table 5.

A. Responsibilities for plate counting and jug harvesting

B. Responsibilities for module disassembly and sample analysis

Coordinator: F. J. de Serres

First Alternate: B. B. Webber

Second Alternote: W. P. Henry

PERSONNEL	RESPONSIBILITIES
Plate Counts and Data Tabulation	
M. T. Sheppard — Coordinator	
A. P. Teasley P. E. Harris M. T. Sheppard	Colony counts
M. T. Sheppord (I. C. Miller)	Data tabulation
M. T. Sheppard (I. C. Miller)	Preparation of IBM data sheets
Jug Harvesting	
B. B. Webber - Coordinator	
(a) Materials	
W. P. Henry	Sterile H_2O, farceps, trays, fluorescent lamps
L. B. Ralston S. H. Lyon P. N. Gambill	Isolation medium
(b) Harvesting	
W. P. Henry	Data sheet preparation
D. S. Carroll J. S. Wassam	Pour samples - (1) measure tatal volume, (2) dispense 1500-ml aliquots, (3) dispense 10-ml background samples
L. B. Ralston, W. K. Barnett, D. W. Ramey, M.D. Shelby, S. H. Lyon, (E. C. Gourley), P. N. Gambill, L. Oggs	Mutant isolatian
M. T. Sheppard	Background aliquots - (1) label tubes, (2) pH measurements, (3) pick up background aliquats
A. P. Teasley P. E. Harris	Mutant colony storage and subculture
E. C. Gourley (M. D. Shelby)	Jug transport from incubatar
M. T. Sheppard D. S. Carrall	Background colony counts
B. B. Webber	Checkout of data sheets

PERSONNEL	RESPONSIBILITIES
Module Disassembly	
M. T. Sheppard — Coordinator	
M. T. Sheppard	Disassemble modules
P. E. Harris	Prepare suspensions from samples, make 10^{-4} dilutions
I. C. Miller	Dosimeter unloading and readout
A. P. Teasley	Hemacytameter caunts
Plating Materials	
L. Oggs - Coardinator	
L. Oggs P. N. Gambill	Prepare plating medium
S. H. Lyan P. N. Gambill	Color code and label plates
D. S. Carroll E. C. Gourley	Media autoclaving and water bath control
L. B. Ralston D. W. Ramey	Sample inoculatian
M. D. Shelby W. K. Barnett	Incubatian of plates
Jug Materials	
W. P. Henry - Coordinatar	
J. S. Wassom M. D. Shelby W. K. Barnett	Prepare jugs
M. D. Shelby W. K. Barnett	Prepare sorbose salution
D. S. Carroll J. S. Wassam	Jug inoculation (a) sorbose addition and sample inoculatian
M. D. Shelby W. K. Barnett	Jug inoculation (b) jug transpartation
E. C. Gaurley (M. D. Shelby)	Jug inoculation (c) air-flaw regulation

B. Preparation of Biological Samples for Biosatellite B

On August 25, conidia of heterokaryon No. 12 from a silica-gel stock culture were suspended in water and then streaked on the surface of Fries minimal medium solidified with agar in petri plates and incubated 2 days at 35° C. The heterokaryon consists of strain 74-OR60-29A (which contains the markers hist-2, ad-3A, ad-3B, nic-2, inos, and ad-2 and is of mating type A), and strain 74-OR31-16A (which contains the markers al-2, pan-2, and cot and is of mating type A). On minimal medium, only those conidia with at least one nucleus of each type are expected to grow. On August 27, single-colony isolates were made from these plates into flasks, on a surface of solidified minimal medium. The flasks were incubated for 2 days at 35° C, and then at room temperature (about 22-25° C) for 5 more days. Nineteen of these cultures were selected on the basis of their color (bright orange) and, on September 3, conidial harvesting was initiated by the addition of about 10 ml of sterile, cold, polished, Pyrex glass beads (3 mm diameter) to each of the culture flasks. The flasks were shaken to break up the conidial clusters as thoroughly as possible, and water (at 6° C) was added to suspend the mixture of mycelium and conidia. From this time until assembly at Cape Kennedy, the temperature of the biological samples was maintained at about 6° C. The resulting suspensions were filtered through platinum gauze to remove clumps of mycelium and then filtered six times through cotton pads to produce a morphologically uniform suspension of cells. Finally the conidia were collected on Millipore filters and washed three times with water. They were then resuspended in water and the conidial concentration was estimated from hemocytometer counts. This suspension was then diluted, and further adjusted to provide about 2000 ml of a suspension containing approximately 5×10^6 conidia/ml. The hemocytometer counts of 2×10^{-5} ml aliquots of the final suspension were 103, 89, 99, 105, 97, and 98 (with an average of 96.83). The precise estimate of the conidial concentration was therefore 4.84×10^6/ml.

C. Platings and Estimates of Conidial Viability

For estimating the viability of heterokaryotic conidia and general viability, the following procedure was used:

1. One-tenth ml of the suspension was pipetted into 9.9 ml of water to provide a 10^{-2} dilution; then a second such 0.1:9.9 dilution was made from the 10^{-2} dilution to provide a 10^{-4} dilution.
2. Two ml of the 10^{-4} dilution were plated in each of two aliquots of a minimal medium in which only the heterokaryotic conidia form colonies. One ml was plated in each of two aliquots of a supplemented medium which supports the growth of all conidia (both heterokaryotic and homokaryotic).
3. Steps 1 and 2 were repeated so that four minimal plates from two separate dilution series and four supplemented plates from the same two dilution series were prepared.
4. These plates were incubated at 30° C, the minimal plates for 3 days and the supplemented plates for 4 days, and the resulting colonies were counted. The counts were as follows:

Minimal plates	
Dilution series I	188 colonies
	189 colonies
Dilution series II	196 colonies
	199 colonies
Average	193 colonies
Supplemented plates	
Dilution series I	345 colonies
	338 colonies
Dilution series II	346 colonies
	363 colonies
Average	348 colonies

5. The estimated number of colonies/ml of original suspension were then divided by the estimated number of conidia/ml of original suspension giving 19.92% heterokaryotic viability and 71.87% general viability.

D. Preparation of Modules

The conidial suspension was maintained at ice-water temperature and kept suspended by the use of a magnetic stirring bar and stirrer. Ten-ml aliquots were harvested on individual Millipore filters (type RAWP 02500) and these were inserted with dosimeters into the module disks, which were in turn assembled to form the modules as described in the modified standing instruction (ORNL-TM 1734). The 15 modules are listed in the Requisition and Shipping Invoice (DD Form 1149) presented as Table 6. The modules listed as Item Nos. 1 through 6 are flight modules and include the five modules which were inserted into the spacecraft and one backup module. The other modules (listed as Item Nos. 7 through 15) are flight-type modules, but were to be used for the ground-control portions of the experiment. The modules were placed into their sterile stainless-steel boxes on September 4 and transported, packed in ice, to Cape Kennedy on that day. Immediately upon arrival at Cape Kennedy, the couriers (W. K. Barnett and S. H. Lyon) placed the modules in the refrigerator at Hangar S, where they were stored at about 6° C until the time for coupling the housings with the modules.

E. Activities of ORNL Personnel Before, During, and After Flight of Biosatellite B

On August 31, the Neurospora laboratory area was tested for the presence of air-borne microbial contaminants by placing nutrient agar plates just above the door to the transfer room for 1 hr. After incubation, seven yeast and/or bacterial colonies were found on each plate. This figure is near the top of the range for similar tests at ORNL. This is a low level of contamination, and these tests showed that no unusual procedures would be required in the coupling protocol to ensure sterility.

Table 6

SHIPPING CONTAINER TALLY

REQUISITION AND INVOICE/SHIPPING DOCUMENT

SHEET NO. 1 | NO. OF SHEETS | 5. REQUISITION DATE | 6. REQUISITION NO.

1. FROM

NAS-2-1900

2. ~~Oak Ridge National Laboratory, Biology Div., Oak Ridge, Tenn.~~

Transportation Officer, Patrick AFB, Florida
General Electric Co. ETR-Hanger "S"
Cape Kennedy, Florida Attn: J. R. Krepps M/F NAS 2-1900

7. DATE MATERIEL REQUIRED | 8. PRIORITY

9. AUTHORITY OR PURPOSE

J. Glancey/Hemiup Agreement

10. SIGNATURE: Ashton B. Cannon

11. VOUCHER NUMBER AND DATE: CS67-19-161

12. DATE SHIPPED: 9/4/67

b. VOUCHER NUMBER AND DATE: 8/5/67

13. MODE OF SHIPMENT: Hand carried

14. BILL OF LADING NUMBER

15. AIR MOVEMENT DESIGNATOR OR PORT REFERENCE NUMBER

3. SHIP TO -MARK FOR

Same as above

4. ACCOUNTING AND FUNDING DATA

~~80X0108 (64)m2510-R-2100~~

ITEM NO. a	FEDERAL STOCK NUMBER, DESCRIPTION, AND CODING OF MATERIEL AND/OR SERVICES b	UNIT OF ISSUE c	QUANTITY REQUESTED d	SUPPLY ACTION e	TYPE CONTAINER f	CONTAINER NOS. g	UNIT PRICE h	TOTAL COST i
1	47D168798G1, Neurospora Module, S/N 1	ea.	1					
2	" " " " 3	"	1					
3	" " " " 4	"	1					
4	" " " " 11	"	1					
5	" " " " 14A	"	1					
6	" " " " 15	"	1					
7	" " " " I	"	1					
8	" " " " III	"	1					
9	" " " " VI	"	1					
10	" " " " VIII	"	1					
11	" " " " IX	"	1					
12	" " " " X	"	1					
13	" " " " XIII	"	1					
14	" " " " XIV	"	1					
15	" " " " XV	"	1					

16. TRANSPORTATION VIA MATS OR MSTS CHARGEABLE TO

17. SPECIAL HANDLING

18. RECAPITULATION OF SHIPMENT

ISSUED BY	TOTAL CONTAINERS	TYPE CONTAINER	DESCRIPTION	TOTAL WEIGHT	TOTAL CUBE
CHECKED BY					
PACKED BY					
		←	TOTAL	→	

19. RECEIPT

CONTAINERS RECEIVED EXCEPT AS NOTED	DATE	BY	SHEET TOTAL
QUANTITIES RECEIVED EXCEPT AS NOTED	DATE	BY	GRAND TOTAL
POSTED	DATE	BY	20. RECEIVER'S VOUCHER NO.

DD FORM 1 MAR 59 ... REPLACES EDITION OF 1 MAY ... WHICH MAY BE USED

On September 7, between 0227 and 0237 EDT (0637 GMT), the six flight modules (including one backup module) were coupled into flight housings previously sterilized by autoclaving. The control I and II modules (for incubation and irradiation simultaneously with flight) were coupled into flight-type housings between 0233 and 0240 EDT (0640 GMT) and the control III module and spare (for incubation commencing 3 hr after the flight modules) were coupled into housings at 0520-0521 EDT (0921 GMT). The module and housing numbers for the flight and each kind of control experiment are listed with the scheme for jug inoculations in Table 7.

The scheduled launch time was 1504 EDT (1904 GMT). Launch was delayed, however, until 1804 EDT (2204 GMT) because of (1) a required change in the installation of the gravity switch and (2) an apparent malfunction of the hydraulic system in the rocket. At this time the estimated data return for the Neurospora experiment was 100%. On September 9, when it became clear that an early call-down would be desirable because of (1) unfavorable weather conditions in the recovery area and (2) difficulties in transmitting signals effectively to the spacecraft from certain of the tracking stations, the expected data return for the Neurospora experiment was estimated at about 55%. This estimate was based on the assumption that the craft would be recovered in an air-snatch and would be returned to Hickam Field without delay. Both of these conditions were met, with air-snatch occurring at 1915 GMT and arrival at Hickam Field at about 2200 GMT.

The control I and control II modules were uncoupled and refrigerated at ice-water temperature by 2125 EDT and the control III modules were uncoupled and refrigerated by 2313 EDT. These were transported from Cape Kennedy to Oak Ridge on September 10, arriving at ORNL around 1900 EDT. The flight assemblies, with modules still coupled in housings, were refrigerated simultaneously with the control I and II modules and transported to Oak Ridge, leaving Hawaii on September 11 around 2300 HST and arriving at Oak Ridge around 1700 EDT on September 12. Uncoupling of the flight modules was not carried out until September 13 and 14 when the conidia were used in the assay system.

Table 7. Plans for inoculation of jugs with conidia from Biosatellite B experiment

Module Designation	Housing Designation	Test or Flight Position	Filter No.	Arbitrary Treatment No.	Jugs Inoculated	Date of Inoculation (September)
3	SN/9	Flight; aft (unirradiated)	2	1	1, 79, 87, 109, 125, 168	13
			9	2	9, 22, 42, 95, 113, 146	13
XIV	20	Control I; aft (unirradiated)	2	3	30, 75, 111, 129, 160, 167	13
			9	4	3, 46, 64, 91, 132, 150	13
1	SN/4	Flight; "6000 R"	1	5	24, 51, 67, 84, 112, 135, 153, 166	13
			2	6	2, 45, 71, 83, 105, 133, 157, 174	13
			4	20	20, 36, 47, 97, 101, 134, 144, 178	13
			6	7	28, 60, 61, 88, 114, 121, 154, 162	13
			9	8	18, 31, 44, 78, 82, 130, 142, 170	13
VIII	16	Control I; "6000 R"	1	9	16, 34, 52, 70, 108, 140, 151, 163	13
			2	10	4, 37, 55, 68, 86, 124, 158, 172	13
			4	25	21, 54, 80, 98, 120, 136, 147, 180	13
			6	11	13, 25, 53, 72, 96, 107, 145, 179	13
			9	12	15, 29, 41, 74, 94, 117, 128, 161	13
4	SN/2	Control II (constant temperature)	2	13	8, 62, 90, 149	14
IX	48	Control III (lapsed time, controlled temperature control)	2	14	12, 23, 106, 165	14
XV	39	Control III	2	15	11, 33, 104, 127	14
15	SN/1	Flight; "2500 R"	1	16	32, 50, 65, 93, 115, 131, 148, 173	14
			5	17	14, 35, 48, 66, 81, 103, 141, 169	14
			9	18	10, 49, 69, 92, 102, 138, 156, 175	14
14	SN/3	Flight; "1000 R"	1	19	17, 26, 59, 85, 118, 123, 159, 177	14
VI	17	Control I; "2500 R"	1	21	19, 39, 58, 63, 116, 122, 155, 176	14
			5	22	6, 40, 57, 73, 89, 137, 143, 171	14
			9	23	7, 27, 56, 76, 100, 110, 139, 164	14
X	18	Control I; "1000 R"	1	24	5, 38, 43, 77, 99, 119, 126, 152	14
11	SN/8	Flight; "500 R"	Conidia not used in analysis			
III	19	Control I; "500 R"				
1	35	Control II				
XIII	37	Control II				

F. Temperature Profile During the Flight

After being coupled, the Neurospora assemblies were kept at about 70-72° F in the laboratory until the scheduled interface. During the flight, telemetered data indicated that the assemblies in the fore compartment were maintained at a temperature range of 67-69° F, while assemblies in the fore compartment of the control I vehicle were kept at about 69° F. The aft (unirradiated) assembly in the flight experienced a temperature range of 64-66° F, which was 6 to 8 Fahrenheit degrees lower than the 72° F registered by the control I aft assembly during most of the flight period.

G. Completion of Preliminary Portions of the Assay

The flight material arrived at ORNL on September 12 and, as previously scheduled, the conidial platings and jug inoculations were completed on September 13 and 14. The scheme for plating and jug inoculations is presented in Table 7; this also indicates the function of each of the 15 modules in the set used for flight. The plans were prepared with the idea that approximately half of the jugs should be inoculated on September 13 and harvested on September 19, and the other half inoculated on September 14 and harvested on September 20, leaving September 21 to finish any remaining jugs. It was considered desirable to use all of the filters selected from a given assembly on the same day, and to open both a given flight package and its corresponding control package from the control I vehicle on the same day. Late in the day on September 13 it was decided to include an additional filter (No. 4 filter) from each of the "6000-R" assemblies in the analysis (treatments 20 and 25) and so these were also inoculated on September 13. The conidia receiving the highest gamma-radiation exposures in both the flight vehicle and the ground control vehicle (contol I) as well as unirradiated conidia from the flight and control I vehicles were introduced into the assay system for both plating and jug inoculation on September 13. On September 14 controlled-temperature, unirradiated control assemblies (controls II and III) and irradiated conidia which would occupy lower points in the dose-response curve and would provide fewer purple mutants

for analysis were processed. All plates were incubated at 30° C. The minimal and pantothenate-supplemented plates were incubated for 3 days and then stored at 4-10° C; the other plates were chilled and stored at 4-10° C after 4 days of growth. The colonies on all plates were counted on September 18 and on that day random colonies were picked from selected plates for the assay of recessive lethal mutations in adenine-independent heterokaryotic conidia. The jugs were incubated in the dark with bubbling and then harvested on September 19-21. The subculturing of all purple colonies obtained from the jugs was also completed by September 21. The genetic characterization of the purple mutants was begun soon after the jug harvesting was completed, but this is expected to require at least 6 months for completion. The analysis of the jug and plating data will be presented in a later quarterly report.

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