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BIOLOGY DIVISION

NEUROSPORA EXPERIMENT P-1037

QUARTERLY PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

OCTOBER 1 - DECEMBER 15, 1966

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DECEMBER 1967

OAK RIDGE NATIONAL LABORATORY
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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: October 1 - December 15, 1966

Principal Investigator:

Frederick J. de Serres

Coinvestigator:

Brooke B. Webber

Technical Staff:

Earle C. Gourley
David S. Carroll
Robert H. Miller
Ida C. Miller
John W. Wassom
Della W. Ramey
Letha Oggs
Linda B. Ralston
Marilyn T. Sheppard
Paula E. Harris
William P. Henry
Arlee P. Teasley
Mary C. Gibson

Name of Institution:

Biology Division
Oak Ridge National Laboratory

Address:

P. O. Box Y
Oak Ridge, Tennessee 37830

Interagency Agreement:

Purchase Order R-104, Task No. 8

Experiment Proposal No.: P-1037

Research jointly sponsored by the National Aeronautics and Space Administration, and by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

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I. INTRODUCTION

The work completed on the Neurospora experiment in the Biosatellite project through 30 September 1966 was described in a previous document (ORNL-TM-1734), which included the design of the experiment, the development, qualification, and final form of the experimental hardware, dosimetry, storage and anoxia experiments, and biocompatibility tests. During the period 1 October through 15 December 1966 the main areas of activity in the preparation of the Neurospora experiment for flight were (1) the preparation and development of adequate laboratory facilities in hanger S at Cape Kennedy and in the trailers at Hickam Field, (2) field training of personnel (by participation in the gantry exercises) to familiarize them with the tasks required for flight, and (3) biocompatibility tests on the flight hardware.

This report includes the work performed up to the flight readiness period which began on 11 December 1966. Work performed in connection with flight on 14 December 1966, will be covered in the next quarterly report.

II. ASSIGNMENT OF PERSONNEL FOR CAPE KENNEDY OPERATIONS FOR BIOSATELLITE A

A preliminary description of assignments and responsibilities of ORNL personnel (Table 1A) for flights and any tests involving simulation of a flight schedule was prepared and submitted to Ames Research Center in June 1966. A schedule of activities (Table 1B) for a flight readiness period of about 30 days was also included. Many of the assignments were subsequently altered because of changes in personnel, because of additional requirements, or as a result of our experience in the Philadelphia and Cape Kennedy preflight tests. Some of these changes are as follows:

1) M. C. Gibson and A. P. Teasley assumed full responsibility for washing and sterilizing modules, inserting the dosimeters, placing the Neurospora samples onto Millipore filters, and for preparing the modules for transport to the test or launch sites. The transport teams were thus relieved of the responsibility of helping with the module preparation at ORNL.

2) D. S. Carroll and E. C. Gourley assumed major responsibilities for the Cape Kennedy operations, including sterilization of the transfer room, washing and sterilizing housings, sterile coupling of modules with housings at the proper time before flight or tests, and sterile uncoupling after flight or tests. They also provided full-time coverage of the Cape Kennedy laboratory during the flight period.

3) I. C. Miller was stationed at Hickam Field during flight as emergency recipient of Neurospora packages and as backup for B. B. Webber. B. B. Webber helped at Cape Kennedy during the gantry exercises, then went to Hickam Field to serve as experimenters' representative in a Samoan contingency group during flight.

4) Conidial platings after tests or flights were carried out by M. C. Gibson and A. P. Teasley. Jugs were inoculated by M. C. Gibson and I. C. Miller.

5) Alterations were made in the constitution of transport teams.

The above changes are reflected in the actual deployment of personnel (Table 2) in connection with the Biosatellite A flight, the pre-flight exercises, and the meetings at Cape Kennedy, Hickam Field, and Ames Research Center.

III. PRECONDITIONING AND BIOCOMPATIBILITY TESTS WITH 301 HARDWARE

On 28 September 1966, at Hangar S at Cape Kennedy, housings 47D169030GI-1, 47D169030GI-2, 47D169030GI-3, 47D169030GI-4, and 47D169030GI-9 were each autoclaved three times after washing and rinsing, washed and rinsed again, double-bagged, and autoclaved in preparation for biocompatibility testing. Each housing was coupled with a module fully loaded with conidia, and the assemblies with conidia were stored at 76-80°F for about 52 hr. After storage, the modules were removed from the housings and transported to the laboratory at Oak Ridge,

where samples were assayed to determine the survival of the heterokaryotic fraction in each module. These survival estimates were compared with viability estimates from samples of the same conidia plated when the modules were first prepared (26 September). The results are presented in Table 3. These data show that there is very little decrease in survival during the 11-day period between preparation and assay. This 11 day period included 52 hours of storage in the pre-conditioned housings.

IV. 301 GANTRY EXERCISE

The 301 gantry exercise of 11-15 October included a full scale countdown, with biological materials prepared on schedule for a flight vehicle, which was to be assembled and prepared as if for flight on the gantry up to just before the simulated launch time. After simulated launch time, the vehicle was disassembled and the experiment assemblies returned to the experimenters. The control experiments prepared for the 301 gantry test were the same as those planned to accompany flight, namely:

control I — irradiated control in a duplicate of the flight vehicle,

control II — constant temperature control,

control III — variable temperature control, in which the loading sequence and control of temperature to duplicate the temperature of the flight vehicle followed the flight vehicle at an interval of 4 hr.

A set of six flight assemblies (five for the flight vehicle and one spare) and nine flight-type assemblies (five for control I, one each for control II and III, and two spares) were used for the 301 gantry exercises.

A. Conidial survival in unirradiated flight vehicle on the gantry. — Because the temperature conditions were not controlled in the flight vehicle after simulated launch time and because the contents of the flight vehicle were not subjected to a

gamma radiation exposure, the conidia from the assemblies in the flight vehicle were assayed only for survival of the heterokaryotic conidia. The data are presented in Table 4. Although there is little difference in survival between the conidia in the vehicle on the gantry and in the spare kept in Hangar S, there is a considerable decrease in survival in all conidia, a fact most readily attributable to residual module toxicity. A toxic effect of housings is ruled out by the good conidial survival rate in the 301 preconditioning and storage test (section III above). However, the 301 gantry exercise was the first test utilizing the 301 flight modules. Further preconditioning and washing of new flight modules was proposed to eliminate toxicity.

B. Dosimetry for the Neurospora experiment in the 301 gantry exercise. — In the irradiated control experiment (Control I), dosimeters at filter positions 1, 6, and 10 in each assembly were used to estimate exposures at these points. Figure 1 is a calibration curve for this batch of dosimeters irradiated in air with an ^{85}Sr source. Table 5 lists the thermoluminescence readings and gamma radiation exposures (derived from the calibration curve) for individual dosimeters. The exposures are plotted in Fig. 2 on a logarithmic scale against log of distance from the center of the radiation source and are fitted with a regression line, which is then used to estimate an exposure at any distance from the center of the source. This procedure is used because it is expected to minimize the effect of any single aberrant dosimeter or small number of aberrant dosimeters and to give the best exposure estimates based on all the data available. It is obvious from Table 5 that the ranges of exposure estimates at each point are rather large — up to 36% of the mean exposure. Means of improving the precision of the exposure estimates for individual dosimeters are being studied and will be described in a subsequent report.

C. Forward-mutation frequencies in the control portion of the 301 gantry exercise — In Table 6, the forward-mutation frequencies obtained for different samples of conidia are given. Included are six irradiated samples and one unirradiated sample from control I, as well as a sample from control II and one from control III (both unirradiated). In Fig. 3 the data are plotted, with means and 95% confidence limits indicated for the forward-mutation frequencies, as well as with a regression line for log of forward-mutation frequency against log of gamma

radiation exposure. There is good agreement between the results of the 301 irradiated-control experiment and the results from laboratory experiments with 250 kvp X-rays given at dose rates of less than 10 R/min.

V. 302 GANTRY EXERCISE

The main values of the 302 gantry exercise were to check the preparation of the Neurospora experiment and the spacecraft loading procedure under timed conditions and to provide final assurance that necessary operations can be completed with appropriate inspection in the allotted time. Because of the brief time interval between the 302 Gantry Exercise and the scheduled Biosatellite A flight, no attempt was made to assay for forward-mutation frequencies; however, biocompatibility tests were performed on the 302 hardware by plating conidia from a number of the modules in the usual fashion to assay survival. The results of these tests are reported in Table 7. The average heterokaryotic conidial survival is 82.6%, which is sufficiently high for use of the conidia in a forward-mutation experiment.

Table 1 A

Personnel List with Responsibilities for Biosatellite A Flight and Gantry Exercises at Cape Kennedy (prepared June 1966)

PERSONNEL	RESPONSIBILITIES
Flight readiness period	
W. E. Porter	(1) Supervise ORNL operations (2) Attend to contingencies at ORNL (3) Complete check list
A. P. Teasley M. C. Gibson	(1) Advise and assist in cleaning and sterilization of modules and harvesting equipment (2) Prepare conidial samples (3) Assemble and load all flight hardware a. biology b. dosimeters (4) Load assembled modules into module carriers
Three teams to transport fresh conidia in modules at two-day intervals.	
No. 1 - J. S. Wassom M. T. Sheppard	(1) Wash and autoclave module components and harvesting equipment
No. 2 - C. L. Shoemake L. Oggs	(2) Provide assistance, as requested, in harvesting conidia and loading modules
No. 3 - D. S. Carroll L. K. Bensey	(3) Transport modules in carriers to launch site per schedule
No. 4 (Alternates) - E. C. Gourley M. McKinzie	(4) Cooperate with G.E. personnel in washing and sterilizing housings (5) Make sterile coupling of modules to housings (6) Make sterile transfer of module from housing to module carrier (if not flown)

	(7) Transport modules in carrier to ORNL
B. B. Webber I. R. Miller	(1) Supervise ground control experiment (2) Record data (3) Attend to contingencies at launch site
F. J. de Serres	(1) Assume prescribed responsibilities as experimenters' representative (2) Maintain general responsibility for the flight package

Flight and postrecovery period

W. E. Porter	(1) Supervise preparation of materials for biological evaluation (2) Attend to contingencies
A. P. Teasley M. C. Gibson	(1) Prepare materials for biological evaluation a. Plating media b. Sorbose solutions for jugs
Transport teams (see above)	(1) Return unused modules and other equipment to ORNL (2) Disassemble unused modules and clean them for storage
D. S. Carroll E. C. Gourley	(1) Assist in preparation for biological evaluation (Mainly jug preparation)
B. B. Webber I. R. Miller	(1) Maintain ground control experiment; collect pertinent data (2) Make sterile transfers of control modules from housings to module carrier (3) Transport control modules to ORNL for analysis
F. J. de Serres	(1) Proceed to recovery area with module carrier (2) Assume prescribed responsibilities as experimenters' representative (3) Maintain responsibility for flight packages (4) Make sterile transfers of modules from housing to module carrier (5) Transport flight modules to ORNL for analysis

Dosimetry and jug inoculation (1-5 days after flight)

A. P. Teasley M. C. Gibson	(1)	Disassemble ground control and flight hardware
	(2)	Prepare biological material for genetic analysis
	(3)	Assist in jug inoculation
B. B. Webber I. R. Miller	(1)	Complete dosimetry for all ground and flight packages
	(2)	Inoculate jugs
	(3)	Prepare report on dosimetry and ground control experiment
F. J. de Serres	(1)	Select ground control and flight samples for analysis
	(2)	Supervise sample plating and jug inoculation

Survival and mutation induction frequency analysis (7-10 days after flight)

W. E. Porter	(1)	Sample jugs
	(2)	Dispense samples to harvesting teams
	(3)	Record appropriate data
D. S. Carroll	(1)	Complete background colony counts on all jugs
	(2)	Record appropriate data

Three harvesting teams:

Shoemake; Teasley Oggs; Sheppard Bensey; Gibson	(1)	Collect purple colonies (with second person in each team to provide confirmation)
	(2)	Record numbers of mutants per aliquot
B. B. Webber	(1)	Process data to obtain tentative survival and forward mutation frequency estimates

Selection of mutants for further characterization (about 10-11 days after flight)

F. J. de Serres	(1)	Analyze data
	(2)	Select proper mutants for further characterization
Harvesting teams (see above)	(1)	Transfer selected mutant samples to isolation medium

Table 1B

Work Schedule for ORNL Preparation Team and Arbitrary Travel Teams for 28-Day Readiness Period Without Flight

	ORNL Module Preparation Team					Travel Team No. 1	Travel Team No. 2	Travel Team No. 3
1	Plate							
2								
3	Pick	Plate						
4								
5	to 22°C	Pick	Plate					
6								
7		to 22°C	Pick	Plate				
8								Jugs
9			to 22°C	Pick	Plate			Jugs
10	to 5°C							
11	Plate	(W & A)		to 22°C	Pick	W & A		Jugs
12		H & P				(H & P)		Jugs
13	Pick	Plate	(W & A)		to 22°C	to Cape	W & A	Jugs
14			H & P			Cape	(H & P)	Jugs
15	to 22°C	Pick	Plate	(W & A)		Cape	to Cape	W & A
16				H & P		to ORNL	Cape	(H & P)
17		to 22°C	Pick	Plate	(W & A)	W & A	Cape	to Cape
18					H & P	(H & P)	to ORNL	Cape
19	(W & A)		to 22°C	Pick	Plate	to Cape	W & A	Cape
20	H & P					Cape	(H & P)	to ORNL
21	Plate	(W & A)		to 22°C	Pick	Cape	to Cape	W & A
22		H & P				to ORNL	Cape	(H & P)
23	Pick	Plate	(W & A)		to 22°C	W & A	Cape	to Cape
24			H & P			(H & P)	to ORNL	Cape
25	to 22°C	Pick	Plate	(W & A)		to Cape	W & A	Cape
26				H & P		Cape	(H & P)	to ORNL
27		to 22°C	Pick	Plate	(W & A)	Cape	to Cape	W & A
28					H & P	to ORNL	Cape	(H & P)
29	(W & A)		to 22°C	Pick	Plate	W & A	Cape	to Cape
30	H & P					(H & P)	to ORNL	Cape
31		(W & A)		to 22°C	Pick	to Cape	W & A	Cape
32		H & P				Cape	(H & P)	to ORNL
33			(W & A)		to 22°C	Cape	to Cape	W & A
34			H & P			to ORNL	Cape	(H & P)
35				(W & A)		W & A	Cape	to Cape
36				H & P		(H & P)	to ORNL	Cape
37					(W & A)	to Cape	W & A	Cape
38				H & P		Cape	(H & P)	to ORNL
39	(wash for storage)					Cape	to Cape	wash for storage
40						to ORNL	Cape	
41	(wash for storage)					wash for storage	Cape	
42							to ORNL	
43	(wash for storage)						wash for storage	

Table 1 B is a schedule for the ORNL module preparation team and travel teams for a readiness period of 28 days at Cape Kennedy, which could be extended or shortened. Symbols are used for the processes described in standard instructions and are as follows:

JUGS - prepare jugs with medium for inoculation of treated conidia; store at 2-6°C

PLATE - plate conidia from silica gel stock culture

PICK - select colonies and transfer to flasks of agar medium, incubate at 35°C

to 22°C - transfer flasks from 35°C to 22°C; score flasks for spreading growth

to 5°C - transfer flasks to 2-6°C cold room; this is done with backup cultures on day 10. Leftover cultures from one cycle serve as backup cultures for the next cycle in each case thereafter and are stored at 2-6°C between successive H & P days

W & A - assume primary responsibility to wash and autoclave hardware and make other preparations for the following day's harvesting

(W & A) - serve in an advisory and auxiliary capacity in washing and autoclaving

H & P - harvest conidia and package them into modules

(H & P) - serve in an auxiliary capacity with harvesting and packaging

TO CAPE - travel to launch site carrying modules in module carrier at ice water temperature

TO ORNL - travel from launch site to ORNL, with modules if these are to be used again

CAPE - interval of responsibility at launch site; serve in auxiliary capacity for investigators at launch site; assume responsibility for proper cleaning and sterilizing of housings and sterile coupling of modules into housing for mock-ups, tests, or flight

WASH FOR STORAGE - final cleaning of used modules and packing for storage

(Wash for storage) - serve in auxiliary and advisory capacity in above procedure

Modifications or adaptations of the module preparation and travel schedule

SUCCESSFUL LAUNCH - ORNL group would be notified to cease the preparation cycle. The travel teams would return to ORNL to help the ORNL team in preparing the sorbose and other materials needed for jug inoculations. See sheet concerning responsibilities

SINGLE ISOLATED TEST OR MOCK-UP - in module preparation schedule use column 1 through day 10 (backup cultures) and column 2 through day 12 (prime cultures). One travel team would be selected and would use travel team No. 1 schedule through day 13, then staying as long as necessary to complete the test and return the modules to ORNL. Day No. 14 would be the first day of the test.

Table 2. Personnel Deployment Chart for Biosatellite A Flight and Gantry E

G. E. Calendar Week No.	39	40	41	42	43	44	45	46	47
Date	Sept. 25	26 27 28 29 30 Oct. 1 2	3 4 5 6 7 8 9	10 11 12 13 14 15 16	17 18 19 20 21 22 23	24 25 26 27 28 29 30	31 Nov. 1 2 3 4 5 6	7 8 9 10 11 12 13	14 15 16 17 18 19
Event		compat. (301)		gantry (301)					
PERSONNEL									
Principal investigator									
F. J. de Serres									
Co-investigator									
B. B. Webber									
Technical assistants:									
E. C. Gourley									
D. S. Carroll									
R. H. Miller									
I. C. Miller									
Transporting teams:									
No. 1 - J. W. Wassom									
D. W. Ramey									
No. 2 - L. Oggs									
L. B. Ralston									
No. 3 - M. T. Sheppard									
P. E. Harris									
Alt. W. P. Henry									
ORNL operations:									
A. P. Teasley	*		*	* * *					
M. C. Gibson	*		*	* * *					

*Equals date for module preparation; ═══ equals Hickam Field, Honolulu; ——— equals Cape Kennedy; - - - - - equals Ames Research C

xercises

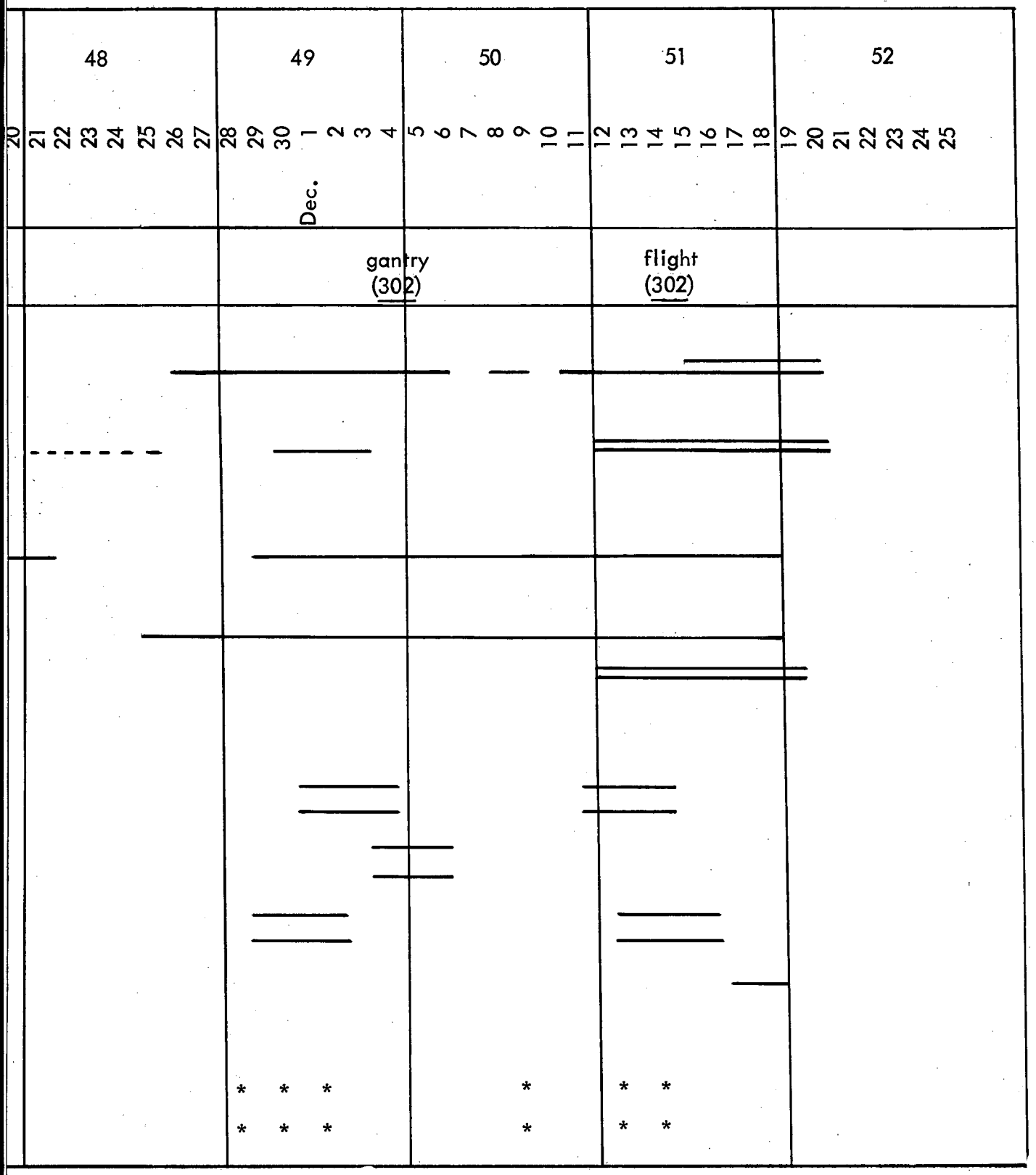

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Table 3. Survival of Heterokaryotic Conidia Stored in 301 Assemblies After Preconditioning (26 September to 7 October). (All data are from platings of unirradiated conidia.)

			Heterokaryotic Conidial Survival	
Housing Designation	Module Designation	Filter Number	Proportion of All Conidia	Percentage of Original Heterokaryotic Proportion (0.1926)
GI-1	A816	1	0.2084	108.2
		10	0.1922	99.8
GI-2	A819	1	0.1897	98.5
		10	0.1692	87.9
GI-3	A818	1	0.1749	90.8
		10	0.1809	93.9
GI-4	A817	1	0.1526	79.2
		10	0.1924	99.9
GI-9	A809	1	0.2175	112.9
		10	0.1645	85.4
	Average Survival		0.1842	95.6

Interval from module preparation to inoculation — 11 days

Table 4. Survival of Heterokaryotic Conidia in the Flight Vehicle in the 301 Gantry Exercise (9-17 October). (All data are from platings of unirradiated conidia.)

Module and Housing Designation	Position in Flight Vehicle	Filter Number	Heterokaryotic Conidial Survival: Proportion of All Conidia	Heterokaryotic Conidial Survival: Percentage of Original Heterokaryotic Proportion (0.1684)
GI-1	6000 R	1	0.1051	62.4
GI-1		10	0.0617	36.6
GI-2	2500 R	1	0.1300	77.2
GI-2		10	0.1070	63.5
GI-3	1000 R	1	0.1067	63.4
GI-3		10	0.1209	71.8
GI-4	500 R	1	0.1253	74.4
GI-4		10	0.0961	57.1
GI-9	Control	1	0.1160	68.9
GI-9		10	0.0853	50.7
GI-	Spare*	1	0.1263	75.0
GI-		10	0.1038	61.6
Average Survival in vehicle			0.1054	62.6
Average Survival in spare*			0.1151	68.3

Interval from module preparation to inoculation — 8 days

* Spare stored in Hanger S Laboratory in Control Area.

Table 5. Thermoluminescence Readings and Corresponding ^{85}Sr Gamma Radiation Exposures (from Calibration Curve) for Dosimeters in the Irradiated Control Portion of the 301 Gantry Exercise

Module Designation and Position in Ground Control Craft	Filter Position	Distance (cm) from Radiation Source to Filter Position	Thermoluminescence Reading	Gamma Radiation Exposure (Roentgens)	
				Separate dosimeters	Mean
IX 36 (6000 R)	1	6.12	1078	7000	6600.00
			927	5800	
			1078	7000	
	6	7.67	489.5	3400	3383.33
			521.0	3600	
			432.8	3150	
	10	8.91	368.6	2750	2916.67
			426.0	3100	
			395.0	2900	
VI 38 (2500 R)	1	9.67	296.6	2300	2433.33
			302.8	2350	
			349.4	2650	
	6	11.22	257.2	2050	1733.33
			172.0	1500	
			191.6	1650	
	10	12.46	134.2	1250	1470.00
			178.8	1580	
			177.8	1580	
A817 (1000R)	1	15.10	99.6	1000	970.00
			98.0	980	
			92.0	930	
	6	16.65	70.0	750	710.00
			65.0	690	
			65.1	690	
	10	17.89	57.4	620	653.33
			60.8	650	
			64.8	690	
A818 (500R)	1	20.94	45.0	490	403.33
			38.1	420	
			27.4	300	
	6	22.49	29.0	320	351.67
			36.4	410	
			31.4	325	
	10	23.73	31.0	345	301.67
			22.3	240	
			29.0	320	

Table 6. Survival of Heterokaryotic Conidia and the Forward-mutation Frequencies for the Control Portion of the 301 Gantry Exercise

Control Series	Housing Designation	Module Designation	Filter Position	Nominal Distance from ^{85}Sr Source (cm)	Gamma Radiation Exposure (Roentgens)	Plating Data on Survival of Heterokaryotic Conidia		Jug Data			
									Survival of Heterokaryotic Conidia		
						Proportion of all Conidia	Percentage of Original Heterokaryotic Proportion (0.1684)	Jug Numbers	Average Porportion of all Conidia	Percentage of Original (0.1684)	Forward Mutation Frequencies
I	16 (6000 R)	IX 36	1	6.12	6326	0.1637	97.2	1-4	0.1017	60.4	91.4
I	16 (6000 R)	IX 36	5	7.36	4300	0.1244	73.9	5-8	0.0922	54.8	61.6
I	17 (2500 R)	VI 38	1	9.67	2318	0.1333	79.2	9-12	0.0819	48.6	47.0
I	17 (2500 R)	VI 38	5	10.91	1750	0.1235	73.3	13-16	0.0994	59.0	27.7
I	18 (1000 R)	A817	1	15.10	871	0.1281	76.1	17-20	0.1033	61.3	15.0
I	19 (500 R)	A818	1	20.94	425	0.1143	67.9	21, 23-25	0.0915	54.3	6.4
I	20 (O R)	A819	1	Control	0	0.1476	87.6	26-30	0.1284	76.2	1.1
II	37	A816	1	Control	0	0.1551	92.1	31-35	0.1176	69.8	0.0
III	39	VIII (35)	1	Control	0	0.1620	96.2	36-41	0.1228	72.9	0.0

Table 7. Survival of Heterokaryotic Conidia in 302 Gantry Exercise (30 November — 7 December). (All Data are from Platings of Unirradiated Conidia.)

Housing Designation	Module Designation	Filter Number	Location in Test	Heterokaryotic Conidial Survival: Proportion of all Conidia	Heterokaryotic Conidial Survival: Percentage of Original Heterokaryotic Proportion (0.1813)
GI-5	GI-3	1	gantry vehicle	0.1481	81.7
GI-27	GI-4	1	gantry vehicle	0.1577	87.0
GI-32	GI-11	1	gantry vehicle	0.1482	81.7
GI-10	GI-15	10	gantry vehicle	0.1685	92.9
GI-6	GI-19	1	gantry vehicle	0.1704	94.0
GI-8	GI-17	1	gantry spare	0.1351	74.5
GI-35	VIII	1	control spare	0.1250	68.9
GI-17	GI-13	1	control vehicle	0.1351	74.5
GI-37	A819	1	control box (III)	0.1600	88.3
	Average Survival			0.1498	82.6

Interval from module preparation to inoculation — 6 or 7 days

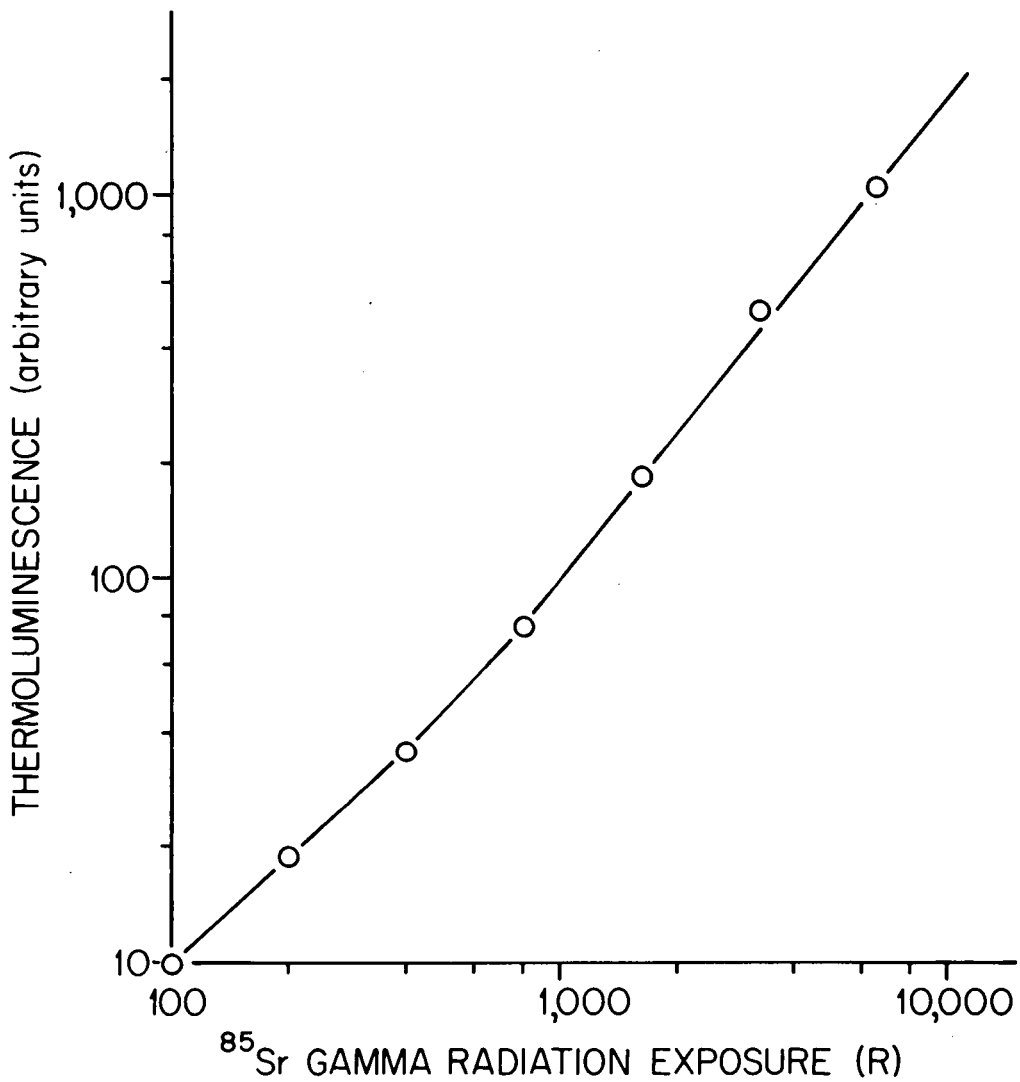

Figure 1. Calibration curve for lithium fluoride teflon disk dosimeters (lot No. 164144) used in 301 and 302 gantry exercises.

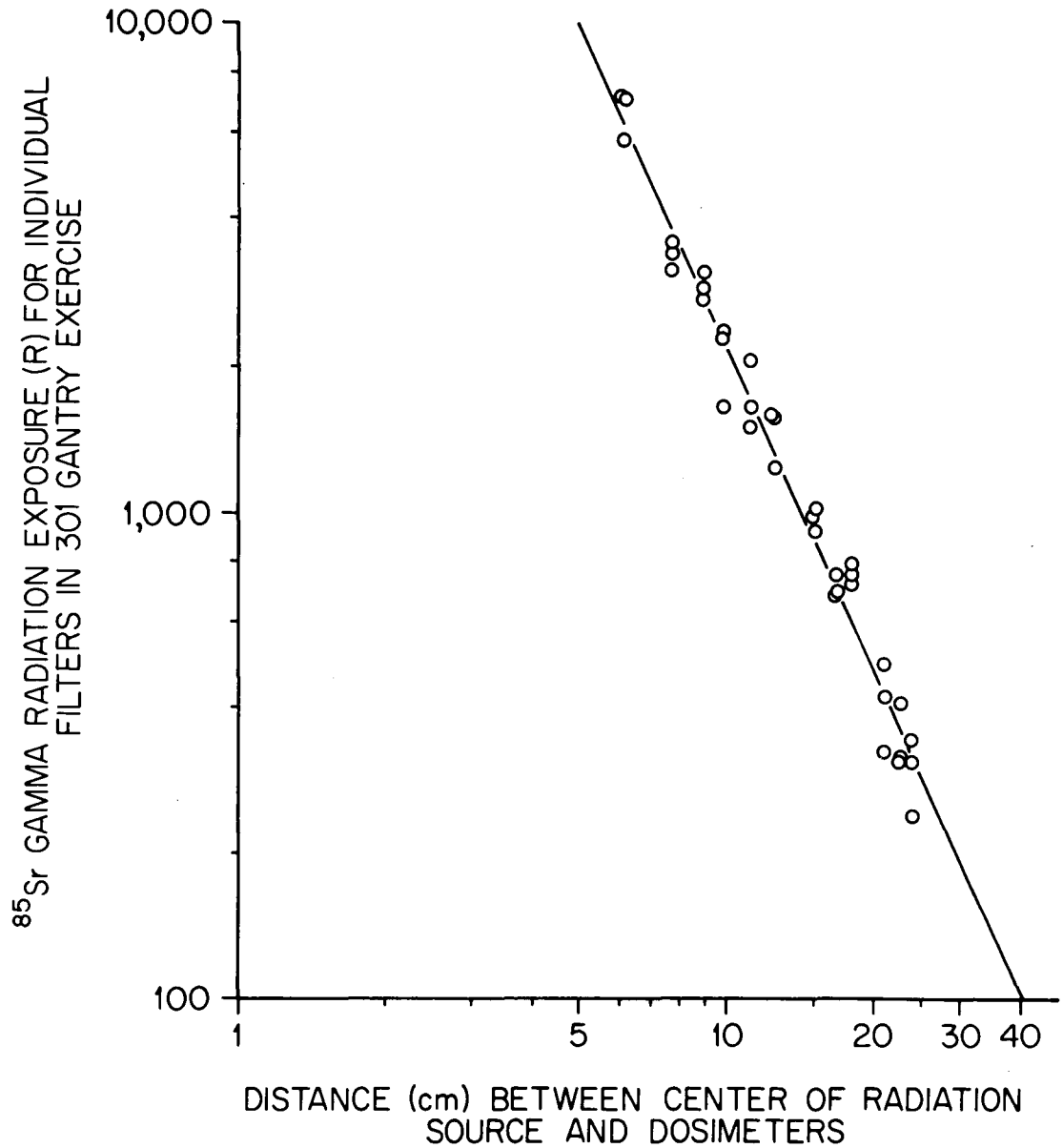

Figure 2. Exposures and positions of dosimeters in 301 gantry exercise plotted to permit extrapolation of exposures to each of the sample positions.

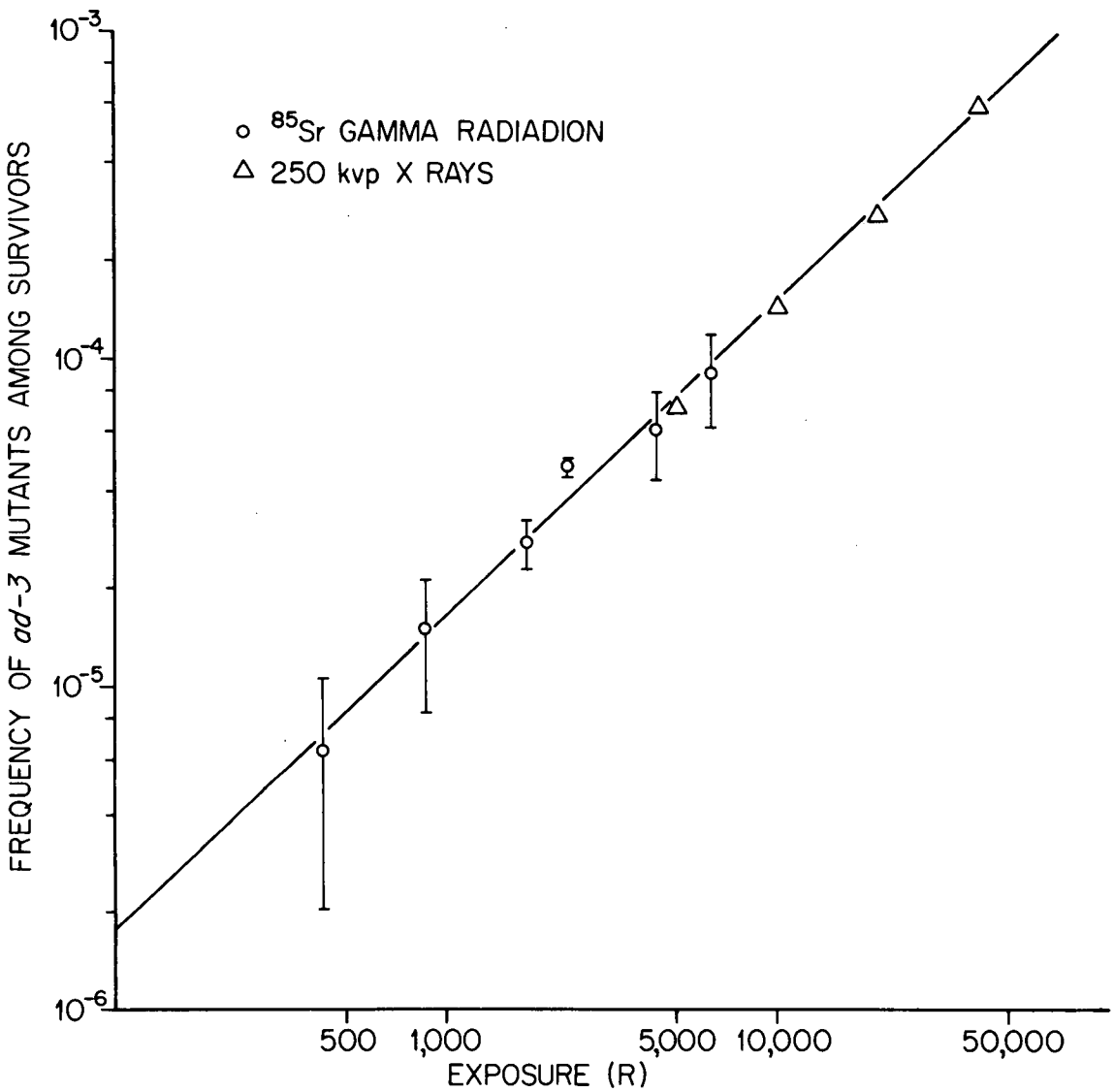

Figure 3. Forward mutation frequency plotted against radiation exposure in (0) irradiated control experiment from 301 gantry exercise and (Δ) a laboratory experiment with 250 kvp X-rays. Regression line and 95% confidence limits are indicated for the gantry exercise data.

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