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QUARTERLY PROGRESS REPORT
TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

January 1 - March 31, 1967

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BIOLOGY DIVISION

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OCTOBER 1967

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 Radiation in Combination with Weightlessness on Habrobracon

For the Period: January 1 - March 31, 1967

Principal Investigator:

R. C. von Borstel

Coinvestigators:

Roger H. Smith

Anna R. Whiting

Robert L. Amy
Department of Biology
Southwestern University
Memphis, Tennessee

Sohei Kondo
Faculty of Medicine
Osaka University
Osaka, Japan

D. S. Grosch (supported by NASA Research Grant No. NsG678)
Department of Genetics
North Carolina State University
Raleigh, North Carolina

Technical Staff:

Katherine T. Cain

Margaret L. Haye

Martha S. Jones

Joan W. Reel

Consultants:

M. B. Baird
Department of Biology
University of Delaware
Newark, Delaware

Mary Lou Pardue
Department of Biology
Yale University
New Haven, Connecticut

Diana B. Smith
Biometrics and Statistics
Oak Ridge National Laboratory
Oak Ridge, Tennessee

L. R. Valcovic
Department of Genetics
North Carolina State University
Raleigh, North Carolina

Name of Institution:

Biology Division
Oak Ridge National Laboratory

Address:

P. O. Box Y
Oak Ridge, Tennessee 37830

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CONTENTS

I. INTRODUCTION

R. C. von Borstel Roger H. Smith

In this report we present the dominant lethality data for sperm from the ground-based control of the 301 walk-thru and from the ground-based control of the Biosatellite flight. No analyses are presented. Here we give only the reduced data because the dosimetry analyses of the spacecraft at present are not thorough enough to enable us to carry out curve and dose-action analyses on the Habrobracon results.

Toshiba glass-rod dosimetry was carried out on the ground-based control in the 301 and 302 walk-thrus and the ground-based control for the Biosatellite. Data from these analyses are compared with data from the LiF dosimetry provided by Dr. John E. Hewitt from Ames Research Center. These comparisons reveal the necessity for more measurements.

II. SURVIVAL OF HABROBRACON MALES AND DOMINANT LETHALITY INDUCED IN SPERM IN THE GROUND-BASED CONTROL OF THE WALK-THRU TEST FOR THE 301 VEHICLE

Roger H. Smith
Anna R. Whiting
R. C. von Borstel
L. R. Valcovic
M. B. Baird
Katherine T. Cain
Joan W. Reel
Martha S. Jones
Margaret J. Lane

The purpose of this experiment was to obtain data during a complete dress rehearsal on the backup (301) Biosatellite vehicle. The artificially scheduled launch was held at 1440 hr on 12 October 1966. Following the test we examined Habrobracon for their survival and measured dominant lethality induced by ^{85}Sr delivered over a 65-hr period. This was to mimic the conditions of the actural flight. The analysis was carried out only on animals used in the ground-based control since only this part of the experiment was carried through to completion. The 301 flight vehicle was not used beyond the time of the artificially scheduled launch.

Results

Survival — Table 1 summarizes the survival of *lemon* males within each module position. Low survival values occurred in the center layer of each module of the controls. This was probably due to a partial crowding effect on the center layer by the outer and inner layers. The Habrobracon males were about a week old at the onset of the experiment, and we reasoned that the deaths in this position could be reduced in future tests if younger animals were used.

Dominant Lethality — The *lemon* males from each dose level were mated to virgin females of the Raleigh strain. Eggs were counted and hatchability recorded. When the hatchabilities were taken, the time of death of the dead embryos was also

Table 1

Survival of males in the different packages

Nominal Exposure (R)		Module Position	No. Wasps	No. Survivors	Survival
4000		UL*	20	20	1.00
		LR†	20	20	1.00
2000		UL	20	20	1.00
		LR	20	20	1.00
1000		UL	20	16	0.80
		LR	20	19	0.95
500		UL	20	17	0.85
		LR	20	16	0.80
Control	inner	UL	20	17	0.85
	middle		20	9	0.45
	outer		20	17	0.85
	inner	LR	20	16	0.80
	middle		20	9	0.45
	outer		20	18	0.90

*UL = upper left module

†LR = lower right module

Table 2

Survival and stages of death during different periods of development after irradiation of Habrobracon sperm

Package Number or Condition	Nominal Exposure (R)	No. Eggs	No. Hatched	Embryo 1	Embryo 2	Embryo 3A	Embryo 3B	Embryo 4A	Embryo 4B	Embryo 5	Larva N	Larva E	Larva M	Larva L	Larva S	Pupa P	Pupa E	Pupa M	Pupa L	Adult Survival ♂	Adult Survival ♀
1	4000	1053	483	540	0	9	1	1	15	4	7	10	3	7	10	38	20	29	0	258	101
2	2000	1028	663	332	0	10	1	3	17	2	9	16	6	11	12	35	25	31	3	257	258
3	1000	959	731	203	0	10	0	1	9	5	10	18	3	1	9	38	17	38	6	268	323
4	500	1001	868	105	1	12	1	1	10	3	2	20	1	14	14	68	19	51	2	236	441
Control	0	357	340	8	0	4	0	0	5	0	5	11	4	3	2	11	3	10	0	104	187
Virgin females	0	2876	2809	16	2	24	5	2	12	6	18	34	12	23	68	160	35	110	14	2335	0

analyzed. The complete data for this, and the data on all stages of death up to the adult, are shown in Table 2.

From the adult survivors the frequency of fertilized eggs could be computed (Atwood, von Borstel, and Whiting, 1956; von Borstel and Rekemeyer, 1959). Using these data, the fraction of surviving diploid eggs (and thus the sperm not containing dominant lethal mutations) could be computed. These frequencies are given in Table 3 for three of the critical stages of development.

Table 3

Dominant lethality frequencies from the ground-based control for the 301 walk-thru test of the Biosatellite experiment

Package Number or Condition	Nominal Exposure (R)	Fraction of Surviving Diploids		
		to blastula (1.0-Type I deaths)	to larva (hatchability)	to adult (eclosability)
1	4000	0.268	0.235	0.137
2	2000	0.536	0.497	0.363
3	1000	0.680	0.650	0.514
4	500	0.854	0.822	0.621
Control	0	0.968	0.939	0.817

Discussion

This is the fourth test where both survival and dominant lethality measurements were taken for males under conditions approximating the actual radiation conditions of the flight. The data are in accord with data from the previous tests (von Borstel et al., 1967; von Borstel and Goins, 1967; Smith et al., 1967).

Only the nominal exposure is given for these tests. The dosimetry experimentation has not been completed yet. It is expected to yield the dose of radiation delivered to the Habrobracon (see the chapter in this volume on radiation conditions for preliminary estimates of the exposures). At this time the exposures are not known to within an accuracy of 20%.

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III. SURVIVAL OF HABROBRACON MALES AND DOMINANT LETHALITY INDUCED IN SPERM IN THE GROUND-BASED CONTROL FOR BIOSATELLITE A

R. C. von Borstel
Roger H. Smith
Anna R. Whiting
L. R. Valcovic
M. B. Baird
Katherine T. Cain
Joan W. Reel
Martha S. Jones
Margaret J. Lane

This report is a summary of data from the ground-based control for Biosatellite A which was launched at 1440 hr on 14 December 1966. The radiation was from a ^{85}Sr source and was delivered over a 65-hr period.

Results

Survival — The survival of *lemon* males within each module position is listed in Table 1. Survival is uniformly high. The males that were used in this test were no older than 30 hr when loaded into the modules and were approximately 30–48 hr old when the irradiation began.

Dominant Lethality — The surviving *lemon* males were mated to virgin females of the Raleigh strain. The numbers of their offspring dying at different stages of development and the numbers of each sex surviving are listed in Table 2.

The data from surviving adults can be used to compute the frequencies of dominant lethal mutations induced at each exposure of radiation (von Borstel and Rekemeyer, 1959). These frequencies are given in Table 3 for three of the critical stages of development.

Discussion

Only the nominal exposures of radiation are listed. The actual exposure received has not yet been accurately determined.

Table 1

Survival of males in the different packages

Control Number	Package Number	Nominal Exposure (R)	Module Position	No. Wasps	No. Survivors	Survival
I	1	4000	UL*	21	21	1.00
			LR†	20	20	1.00
I	2	2000	UL	20	20	1.00
			LR	20	20	1.00
I	3	1000	UL	20	20	1.00
			LR	20	20	1.00
I	4	500	UL	20	19	0.95
			LR	20	20	1.00
I	5	pre-2000	UL inner	20	20	1.00
		0	middle	20	20	1.00
		0	outer	20	20	1.00
		pre-2000	LR inner	20	20	1.00
		0	middle	21	21	1.00
		0	outer	20	19	0.95
II	35	pre-2000	UL	20	20	1.00
		0	LR	20	20	1.00
		0	LL	20	20	1.00
II	40	0	UL	20	20	1.00
		0	UR	20	20	1.00
		0	LR	20	20	1.00
		0	LL	20	20	1.00
II	43	0	UL	20	20	1.00
		0	UR	20	20	1.00
		0	LR	20	20	1.00
		0	LL	20	19	0.95
III	41	pre-2000	UL	20	20	1.00
		0	LR	20	20	1.00
III	42	0	UL	20	20	1.00
		0	UR	20	20	1.00
		0	LR	20	20	1.00
		0	LL	20	20	1.00
III	36	0	UL	20	19	0.95
		0	UR	20	20	1.00
		0	LR	20	19	0.95
		0	LL	20	20	1.00

*UL = upper left module

†LR = lower right module

Table 2

Survival and stages of death during different periods of development after irradiation of Habrobracon sperm

Control Number	Package Number	Nominal Exposure (R)	Module Position	No. Eggs	No. Hatch	Embryo							Larva					Pupa				Adult Survival	
						1	2	3A	3B	4A	4B	5	N	E	M	L	S	P	E	M	L	♂	♀
I	1	4000	UL*	785	390	370	0	2	0	2	17	4	10	7	5	5	3	13	9	16	21	222	79
			LR†	1163	573	543	0	22	3	3	11	8	15	5	7	11	1	4	10	5	36	385	94
I	2	2000	UL	1139	736	375	0	11	4	2	10	5	14	13	6	12	7	9	14	10	52	353	246
			LR	796	495	280	1	5	1	0	8	6	12	18	5	3	3	15	3	18	23	217	178
I	3	1000	UL	763	609	127	0	9	0	3	12	3	7	21	4	6	4	14	13	17	31	257	235
			LR	1071	833	196	3	15	0	0	20	4	24	16	6	1	3	10	6	17	36	348	366
I	4	500	UL	1208	1047	126	0	14	6	1	13	1	21	24	8	2	4	20	8	32	63	385	480
			LR	1037	898	100	1	20	2	1	13	2	17	23	7	5	2	13	10	15	55	314	437
			UL																				
I	5	pre-2000	inner	986	669	290	1	13	1	0	10	2	10	21	3	4	7	11	7	12	24	320	250
		0	middle	707	679	11	1	9	1	0	5	1	3	19	1	3	1	34	8	11	51	204	344
		0	outer	769	746	11	0	7	2	1	2	0	19	16	6	4	6	14	9	10	68	226	368
			LR																				
		pre-2000	inner	1046	697	317	1	16	2	2	11	0	5	24	4	4	4	13	11	23	26	361	222
		0	middle	941	918	11	0	4	0	1	5	2	10	21	6	3	6	14	9	22	42	298	487
		0	outer	883	853	10	0	9	0	2	3	6	8	23	6	2	6	11	8	16	44	273	456
II	35	pre-2000	UL	944	659	269	2	6	1	1	4	2	9	10	2	6	6	9	8	22	19	341	227
		0	LR	788	765	7	0	6	2	0	4	4	13	16	2	2	0	8	19	39	58	246	362
		0	LL	1112	1080	14	0	12	0	1	2	3	16	17	10	4	7	32	14	31	96	310	543
	40	0	LL	678	657	14	1	1	1	0	3	1	1	13	2	14	9	25	19	47	47	183	297
III	41	pre-2000	UL	856	600	238	4	4	3	1	5	1	5	21	0	3	8	11	16	28	29	262	217
		0	LR	1001	973	16	0	6	0	1	3	2	9	15	4	6	10	34	20	54	50	299	472
Virgin Females		0		4631	4450	61	1	84	15	2	9	9	27	57	23	27	44	106	67	148	223	3728	—

*UL = upper life module

†LR = lower right module

Table 3

Dominant lethality frequencies from Control I of the ground-based control series of Biosatellite A

Package Number or Condition	Nominal Exposure (R)	Fraction of Surviving Diploids		
		to blastula (1.0-Type I deaths)	to larva (hatchability)	to adult (eclosability)
1	4000	0.244	0.200	0.145
2	2000	0.474	0.449	0.346
3	1000	0.711	0.665	0.555
4	500	0.844	0.807	0.666
Control	0	0.986	0.975	0.766
Preirradiated control	2000	0.511	0.488	0.409

Literature Cited

von Borstel, R. C., and M. L. Rekemeyer, 1959. Radiation-Induced and Genetically Contrived Dominant Lethality in Habrobracon and Drosophila. Genetics, **44**: 1053-1074.

IV. RADIATION CONDITIONS FOR THE HABROBRACON PACKAGES IN THE GROUND-BASED CONTROLS OF THE FIRST BIOSATELLITE FLIGHT

R. C. von Borstel
J. E. Hewitt
Katherine T. Cain
Nanci L. Badger
Diana B. Smith

Three Toshiba glass rods were used in each of the four individual modules in every Habrobracon package in the ground-based control experiments for the backup (301) vehicle walk-thru (12-15 October 1966), the flight (302) vehicle walk-thru (2-5 December 1966), and the actual flight (14-17 December 1966). In addition to the glass-rod dosimeters, LiF encased in plastic tubes was used for measurement of the general exposure to each of the packages. The glass-rod dosimetry is the responsibility of the group at Oak Ridge, and the LiF dosimetry is the responsibility of the group at Ames Research Center.

The dosimetry measurements for these experiments allowed us to make repeated comparisons among the same modules and packages, and also permitted a check of the reliability of the two types of dosimetry for the same packages.

Cross-Calibration Measurements

Calibration curves for both the Toshiba glass rods and the LiF powder were based on Victoreen ionization chamber measurements. The ionization chambers from the Oak Ridge National Laboratory and Ames Research Center were calibrated separately at the National Bureau of Standards with ^{137}Cs γ-radiation. A ^{60}Co γ-radiation calibration curve for the Toshiba glass rods was then constructed (Fig. 1) from experiments carried out at Oak Ridge. Since the source to be used in the Biosatellite is of ^{85}Sr, ^{85}Sr γ-radiation calibration curves for both LiF and glass rods were made at Ames Research Center. The glass-rod data from this series of measurements falls on the ^{137}Cs γ-radiation curve (Fig. 1).

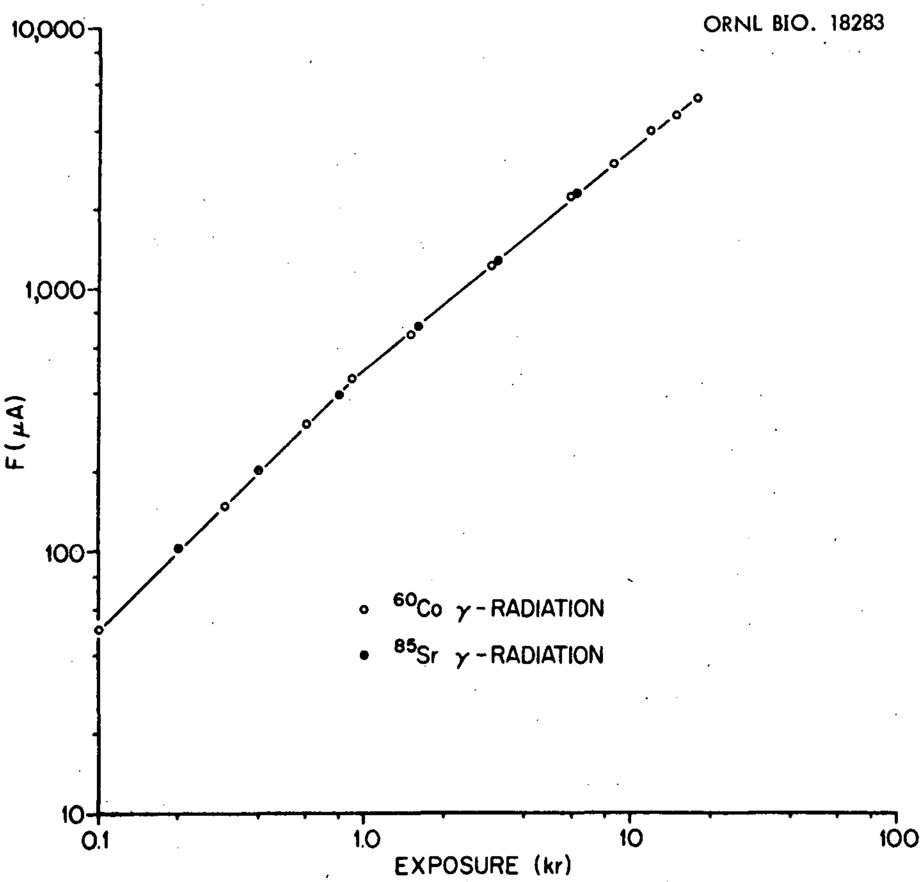

Fig. 1. The Dose-Fluorescence Curve for Toshiba Glass Rods Exposed to ^{60}Co and ^{85}Sr γ-Radiation. F is the designation for the fluorescence in microamperes on the Toshiba reader. The ^{60}Co γ-radiation calibration curve was prepared by Sohei Kondo.

LiF and Glass-Rod Measurements of Exposures to the Habrobracon Packages

The glass rods and the LiF are displaced slightly from the position of the Habrobracon. A correction must be made for both types of dosimetry to obtain estimates of the exposures to the animals. For the LiF the largest correction has to be made for the geometric configurations, and for the glass rods the largest correction has to be made for low-energy radiation from scattering. Neither of these has been achieved satisfactorily.

LiF — The LiF data from the three tests at Cape Kennedy are listed in Table 1.

Table 1

Comparison of exposures to LiF in the front and back tubes of the Habrobracon packages in the ground-based control experiments

Nominal Exposures (R)	301 Walk-Thru Exposures (R)			302 Walk-Thru Exposures (R)			Flight Exposures (R)			P*
	Front Tube	Back Tube	Front /Back	Front Tube	Back Tube	Front /Back	Front Tube	Back Tube	Front /Back	
4000	4650	2040	2.28	5400	2520	2.14	5830	2450	2.38	<0.005
2000	2180	1140	1.91	2700	1400	1.93	2750	1430	1.92	0.98
1000	1180	722	1.63	1470	938	1.57	1470	910	1.56	0.79
500	530	361	1.47	690	487	1.42	700	450	1.56	0.55

*The probability that the ratio of the readings of LiF in the front and back tubes is the same at any position in the three experiments.

By obtaining a ratio for the LiF measurements from the front and back tubes at any one position the readings from test to test can be compared. The reliability of the method can thus be estimated independently from the actual exposures, which indeed did vary from experiment to experiment. It can be seen that these ratios are consistent from test to test except at the 4000 R nominal exposure where the P value for similarity is <0.005. Since the front to back ratio was stable from test to test at the 2000 R nominal exposure, data taken at this position were selected to further examine the aberrant front to back ratios at 4000 R. The ratio of the 4000 R to 2000 R nominal exposures were made for comparison of the LiF readings for each test in the front tubes (P = 0.12) and the back tubes (P = 0.53). These comparisons suggest that the discrepancy for the 4000 R nominal exposure is associated with one or two of the readings of LiF from the front tubes.

Provisional estimates of the actual exposures to the Habrobracon were made by dose-square calculations. Only the data obtained from LiF in the front tubes were used in these calculations. The estimates are shown in Table 2.

Table 2

Computed exposures to Habrobracon based on LiF measurements from the anterior tube (from Hewitt, Fenrick, and Badger, 1967)

Nominal Exposure (R)	301 Walk-Thru (R)	302 Walk-Thru (R)	Biosatellite A Ground Control I (R)
4000	3440	4000	4310
2000	1910	2380	2420
1000	1070	1340	1340
500	494	642	650
0	1.0	0.7	0.8
0 (back tube)	1.0	0.8	0.8

Glass Rods — It is not possible at this time to make precise estimates from the glass-rod measurements of the exposures to the animals, since the scattering characteristics of these mock-ups appear to be different from the mock-up for the biocompatibility test used in June 1966 (Kondo and von Borstel, 1967). Unfortunately, new calibrations must be made. Further, the geometric corrections for each module have not been established by experiment. It is necessary that this be done experimentally because variability of the incident doses exists among modules as shown by the glass-rod data (Tables 3, 4, and 5). This variability was extreme in the nominal 500-R exposure position in the 301 walk-thru ground-based control (Table 2). It was assumed that the large variation among the nominal 500 R modules in this experiment was because the right side of the package (as looked at from the source) was partially in shadow. By twisting the package away from the shadow, the right and left sides of the package received more nearly equal exposures (Tables 3 and 4) in the later tests.

Table 3

Fluorescent readings of the Toshiba glass rods in the ground-based control of the 301 walk-thru on 12-15 October 1966 (uncorrected for scattered radiation)

Nominal Exposure (R)	Module Position	2F* Average Module	2F Average Horizontal (lower and upper)	2F Average Vertical (right and left)	2F Average Package	Average Package Exposure (R)
4000	LR†	2814	2800	2906	2926	3530
	LL‡	2786		2947		
	URδ	2997	3052			
	UL¶	3108				
2000	LR	1540	1557	1626	1626	1700
	LL	1574		1626		
	UR	1713	1696			
	UL	1678				
1000	LR	1007	1007	1037	1032	1050
	LL	1007		1026		
	UR	1068	1057			
	UL	1046				
500	LR	445	478	482	504	504
	LL	511		526		
	UR	519	530			
	UL	540				
0	LR	2.5	2.6	2.6	2.6	2.6
	LL	2.7		2.6		
	UR	2.6	2.6			
	UL	2.5				

*F is the designation for the fluorescence measurement shown for the ordinate in Fig. 1.

†LR = lower right

‡LL = lower left

δUR = upper right

¶UL = upper left

Table 4

Fluorescent readings of the Toshiba glass rods in the ground-based control of the 302 walk-thru on 2-5 December 1966 (uncorrected for scattered radiation)

Nominal Exposure (R)	Module Position	2F* Average Module	2F Average Horizontal (lower and upper)	2F Average Vertical (right and left)	2F Average Package	Average Package Exposure (R)
4000	LR†	3560.7	3572.3	3646.5	3677.7	4700
	LL‡	3583.9		3708.9		
	UR§	3732.3	3783.2			
	UL¶	3834.0				
2000	LR	2018.1	2019.9	2041.9	2038.4	2230
	LL	2021.7		2034.9		
	UR	2065.8	2056.9			
	UL	2048.1				
1000	LR	1225.3	1212.6	1242.2	1232.5	1270
	LL	1199.9		1222.8		
	UR	1259.1	1252.4			
	UL	1245.7				
500	LR	619.8	614.9	628.3	625.9	625.9
	LL	610.1		623.4		
	UR	636.8	636.8			
	UL	636.8				
0	LR	1.7	1.8	1.7	1.7	1.7
	LL	1.8		1.7		
	UR	1.7	1.6			
	UL	1.5				

*F is the designation for the fluorescence measurement shown for the ordinate of Fig. 1.

†LR = lower right

‡LL = lower left

§UR = upper right

¶UL = upper left

Table 5

Fluorescent readings of the Toshiba glass rods in the ground-based control of the Biosatellite flight experiment of 14-17 December 1966 (uncorrected for scattered radiation)

Nominal Exposure (R)	Module Position	2F* Average Module	2F Average Horizontal (lower and upper)	2F Average Vertical (right and left)	2F Average Package	Average Package Exposure (R)
4000	LR†	3671.8	3670.9	3710.3	3725.1	4800
	LL‡	3670.1		3739.8		
	UR§	3748.9	3779.2			
	UL¶	3809.4				
2000	LR	2081.8	2081.8	2100.6	2097.5	2310
	LL	2081.8		2094.4		
	UR	2119.4	2113.2			
	UL	2107.0				
1000	LR	1271.7	1266.4	1286.9	1279.2	1320
	LL	1261.2		1271.4		
	UR	1302.1	1291.9			
	UL	1281.7				
500	LR	636.4	633.5	639.8	639.9	639.9
	LL	630.7		640.0		
	UR	643.3	646.3			
	UL	649.3				
0	LR	2.5	2.5	2.3	2.3	1.7
	LL	2.4		2.3		
	UR	2.2	2.2			
	UL	2.2				

*F is the designation for the fluorescence measurement shown for the ordinate in Fig. 1.

†LR = lower right

‡LL = lower left

§UR = upper right

¶UL = upper left

Comparisons were made of the data from measurements of the glass rods in all tests. When the ratios at all nominal doses were taken together no significant difference between tests was indicated (P = 0.46).

LiF and Glass Rod Comparisons — Ratios of LiF and glass-rod measurements at each position were obtained. These are presented in Table 6. It can be seen that

Table 6

Comparison of physical dosimetry measurements on the Habrobracon packages in the ground-based control experiment (uncorrected for geometry and scattered radiation)

Nominal Exposures (R)	LiF Tube Position	301 Walk-Thru Measured Exposures			302 Walk-Thru Measured Exposures			Flight Measured Exposures			P†
		LiF	Glass Rods*	LiF/Glass Rods	LiF	Glass Rods*	LiF/Glass Rods	LiF	Glass Rods*	LiF/Glass Rods	
4000	front	4650	3530	1.32	5400	4700	1.15	5830	4800	1.22	<0.005
	back	2040		0.76	2520		0.54	2450		0.51	<0.005
2000	front	2180	1700	1.28	2700	2230	1.21	2750	2310	1.19	0.217
	back	1140		0.67	1400		0.63	1430		0.62	0.262
1000	front	1180	1050	1.12	1470	1270	1.16	1470	1320	1.11	0.759
	back	722		0.69	938		0.74	910		0.69	0.443
500	front	530	504	1.06	690	625.9	1.10	700	639.9	1.09	0.839
	back	361		0.72	487		0.78	450		0.70	0.473
0		1.0	2.6	0.385	0.75	1.7	0.441	0.8	2.3	0.348	

*Average of rods in four modules (each module averaged separately).

†The probability that the ratios obtained from the LiF and the glass rods are constant from experiment to experiment at any one position.

the P values are particularly low at the position of the 4000 R nominal exposures. Part of the reason for the low P values can be attributed to the fluctuations in the LiF measurements, and part to the changes from mock-up to mock-up in the amount of scattered radiation.

We have seen in the earlier sections that the LiF measurements appear to maintain a higher intrinsic variability than do the glass-rod measurements. It was noted that the largest discrepancy appears in the anterior tube group of measurements at the 4000 R nominal position. This is confirmed by the glass-rod comparisons. Confidence in the system can only be gained by repeated testing under identical conditions. So far the conditions have not been identical in any of the tests.

The lack of identity is seen through shifts in the ratios of the LiF to glass-rod measurements. These are probably the result of scattered radiation changes that occur with the different subassembly masses that were available at each test. Only further testing can tell.

Recommendations

1. To establish a provisional exposure to the Habrobracon, before the next flight a test must be made where LiF will be placed in the chamber where the animals are to be held. This experiment will be used as the guide for the preliminary report on the effect of the flight on the animals.

2. Post-flight dosimetry on the recovered Biosatellite capsule is mandatory to firmly establish the characteristics of the radiation for direct comparison of the animals in the Biosatellite to those in the ground-based control.

3. Scattering determinations must be taken after the flight on both the recovered capsule and the ground-based control mock-up.

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