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QUARTERLY PROGRESS REPORT TO THE NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

October 1 - December 31, 1966

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BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT
TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

October 1 – December 31, 1966

DECEMBER 1967

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee
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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: October 1 – December 31, 1966

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P-1079

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CONTENTS

I. Introduction . . . . . .

II. Survival and Fecundity of Habrobracon Females Under Different Conditions of Feeding Before Starvation . . . . . .

III. Survival of Habrobracon Males Under Different Conditions of Crowding . . . . . .

IV. Measurement of Positions of Habrobracon Packages in the Biosatellite . . . . . .

V. Measurement of the Positions of the Habrobracon Nominal 500-R Package in the Biosatellite and in the Ground-Based Control Prior to Launch . . . . . .

VI. Goddard Space Flight Center Operations for Biosatellite A . . . .

VII. The Flight of Biosatellite A . . . . . .

VIII. Evaluation of Flight-Time Preparations for the Habrobracon (P-1079) Experiment in the First Biosatellite . . . . . .

IX. Standing Instructions and Flight-Time Instructions for the Habrobracon (P-1079) Experiment in the Three-Day Biosatellite . . .

I. INTRODUCTION

R. C. von Borstel Roger H. Smith

This quarterly report is essentially a summary of the different kinds of work that went into preparing the Habrobracon experiment and experimenters prior to launching Biosatellite A. The culmination of all the preparations is the standing and flight-time instructions for the Habrobracon experiment which are presented at the end of the current report.

In-flight operations and post-flight evaluations for the Habrobracon experiment are also presented.

No radiation data on Habrobracon are given in this report even though dominant lethality results were obtained from the 301 walk-thru on 12 October. These will be presented in the next report, where they can be more closely compared with the ground-based control data from the Biosatellite A experiment.

II. SURVIVAL AND FECUNDITY OF HABROBRACON FEMALES UNDER DIFFERENT CONDITIONS OF FEEDING BEFORE STARVATION

M. B. Baird Roger H. Smith

One of the important experimental endpoints in the Habrobracon portion of the Biosatellite experiment is concerned with the differential radiosensitivity of Habrobracon oocytes which are exposed to radiation in different stages of meiotic development. Habrobracon females will produce mature oocytes only when they are feeding upon the host Ephestia larvae. The Habrobracon will only lay eggs on or near the host larvae. If female Habrobracon are allowed to feed on the host larvae for 24 hr and then removed from contact with the host, the female will store oocytes, which are in various stages of meiotic development. If these females are exposed to radiation, the oocytes exhibit a differential response to the radiation. This differential response is determined by allowing the exposed females to lay eggs on the host larvae for different periods of time. Each sequential time period of egg-laying corresponds to oocytes which were stored by the female Habrobracon in an earlier stage of meiotic development. Thus, for the Raleigh (R) strain, after irradiation, the eggs which are laid in the first 4 hr were irradiated while in metaphase I, the eggs laid in the next 4 hr were irradiated in metaphase I and in late prophase I (diakinesis), and the eggs laid in the following 16 hr were irradiated in the diplotene stage of prophase I. Therefore, the eggs laid by irradiated Habrobracon females in the first 24 hr of egg-laying following irradiation represent at least three stages of meiotic development.

It was observed, as a result of testing the females after starvation for a period of time corresponding to the space flight, that few if any eggs are produced in the first 6-8 hr of egg-laying. This reduction may be caused by egg resorption by the starved Habrobracon females, beginning with the most advanced eggs in meiotic development.

It was thought that feeding honey to the females throughout the period of time that the wasps are storing the eggs, prior to the irradiation of the females, might produce an increase in the number of eggs produced in the first 24 hr when egg-laying is resumed.

Materials and Methods

Virgin Habrobracon females (Raleigh) were allowed to feed upon Ephestia larvae for 24 hr at 86°F ± 2°. The females were removed from contact with the host larvae and divided into two groups. One group of 46 females were fed a mixture of honey and water. The second group of 19 were not fed honey. Both groups were incubated at 86°F ± 2° for 18 hr, and then maintained at 70°F ± 3° for 2 hr to simulate the time when the Habrobracon would normally be irradiated.

The Habrobracon were then loaded into precleaned radiation modules according to the standing instructions. Nineteen females were placed in three modules and eight female wasps were placed in a fourth module. The modules were screwed finger tight into Habrobracon brackets and placed at 70°F ± 3°.

After 4 days and 22 hr the Habrobracon were removed from the modules in the manner described in the standing instructions, and the number of Habrobracon surviving in each module was recorded. The Habrobracon were placed individually in 1-1/2 inch Stender dishes and presented with a prestung Ephestia larva. The Habrobracon were transferred to fresh Stender dishes with prestung host larvae at the following intervals: 4 hr, 2 hr, and 18 hr, for a total of three transfers. The number of eggs produced by each female was recorded for each transfer.

Results

The results of this study are presented in Table 1. It is evident from this table that feeding honey to Habrobracon females throughout the period of egg storage does

not increase the number of eggs laid by the females in the first 6 hr of egg-laying following nearly 5 days of starvation. However, the mean number of eggs laid by the females in Transfer III is 4.0 eggs per female for the honey-fed females as compared with 0.9 eggs per female for the females not fed honey. Thus, females which have been fed honey during the period of egg storage produce a greater number of eggs in the last 16 hr of the first day of egg-laying than do females which have not been fed honey.

Table 1

Survival and egg production of Habrobracon females (Raleigh) maintained in radiation modules for four days and twenty-two hours

Module No. and Position	No. of Habro-bracon	No. Surviving	% Survival	Honey-fed*	No. of Eggs Produced		
					Transfer† I	Transfer II	Transfer III
1 Upper left	19	14	74	-	0	0	12
1 Lower right	19	17	89	+	0	0	82
3 Upper left	8	5	62	+	0	0	13
3 Lower right	19	19	100	+	0	0	69

*Honey-fed for 20 hr after removal from Ephestia larvae.
†Hours of egg-laying: I (4 hr), II (2 hr), III (18 hr).

Inspection of Table 1 also indicates that the females which were fed honey in this study had a greater survival than females which were not fed honey, at least in modules with comparable numbers of Habrobracon. The percent survival in the module containing eight Habrobracon was less than any of the other three modules, including the module containing the females which were not fed honey.

Discussion

The results of this study indicate that feeding honey to Habrobracon females during the period of egg storage does not increase the number of eggs laid during the first 6 hr of egg-laying following 4 days and 22 hr of starvation. However, the honey-fed females produce more eggs in the last 16 hr of the first 24 hr of egg-laying than do females which have not been fed honey. Also, the survival of the honey-fed females is greater than the survival of the females which have not been fed honey.

The number of eggs usually laid by Habrobracon females in Transfer III after 4 days of starvation varies from 7-15 eggs per female. The results obtained in this study are much smaller than this range. There are probably two reasons why the females in this study laid so few eggs in Transfer III. First, the females used in this study were old females, having been mailed to Cape Kennedy from the laboratory in Oak Ridge, Tennessee. Old females are not as able to withstand environmental stress as are young ones. Second, the span of time covered by this study is nearly a day longer than is expected for the actual flight. Thus, the females in this study were subjected to nearly 5 days of starvation.

The increase in survival of the honey-fed Habrobracon is to be expected, as the honey-fed Habrobracon were starved for 18 hr less. Also, it is known that honey-fed Habrobracon have a longer life-span than Ephestia-fed Habrobracon. The low survival in the module containing eight Habrobracon may have been a result of increased activity of the Habrobracon in this module because the females were less crowded.

Summary

1. The effect of feeding honey during the 18 hr preceding irradiation on the number of eggs produced by Habrobracon females in the first 24 hr after 4 days and 22 hr of starvation was studied.

2. No increase in the number of eggs laid in the first 6 hr was observed in the honey-fed females over those not fed honey. However, the number of eggs per female produced in the next 16 hr was greater in the group of females that were fed honey.

3. The survival of the honey-fed females was greater than the survival of the females which were not fed honey.

III. SURVIVAL OF HABROBRACON MALES UNDER DIFFERENT CONDITIONS OF CROWDING

M. B. Baird

The modules in the Habrobracon packages had been designed to contain 12 Habrobracon under noncrowded conditions. It was found that an increase in number up to 20 per module did not affect survival (Smith et al., 1967). A study of the survival data from the various tests at General Electric in Philadelphia indicated that survival of the adult Habrobracon in the middle layer of the three-layered control module was lower than survival in the two outer layers. However, this decreased survival in the middle layer was not observed in all of the tests, nor was it observed in all of the control modules when it was observed in a particular test.

This study is first of all an attempt to determine the maximum number of Habrobracon which may be loaded in each module or module layer without causing the Habrobracon to die as a result of overcrowding. Second, this study presents survival of Habrobracon in the middle layer of control modules as compared with survival in the outer and inner layers.

Materials and Methods

The module chamber in which the Habrobracon are contained is cylindrical in shape with an inner diameter of 0.448 inch and a depth of 0.074 inch.

The method utilized in the first experiment was as follows. Twelve Habrobracon radiation modules were cleaned in the manner prescribed in the standing instructions. The modules were loaded with Habrobracon males (Raleigh strain) which were fed overnight on a honey-water mixture. These males had eclosed 6-8 days previously. The number of Habrobracon in each module was 30, 40, or 50. The modules were

screwed finger tight into brackets, and the brackets were maintained at 70°F ± 3°. After 4 days and 2 hr, the modules were unloaded in the manner specified in the standing instructions, and the number of survivors per module was recorded.

In the second experiment, six Habrobracon control modules were cleaned according to standing instructions. The modules were loaded with unfed, freshly eclosed Habrobracon males (Raleigh strain) according to the standing instructions. Each module layer was loaded with 12, 15, 20, 25, or 30 Habrobracon. The number of Habrobracon per module layer was varied from module to module, but not within modules. The modules were screwed finger tight into brackets, and the brackets were maintained at 70°F ± 3°.

After 4 days, the modules were unloaded in the manner specified in the standing instructions, and the number of survivors was recorded for each module layer.

Results

The results of the first experiment involving the radiation modules are presented in Table 1. Although the percentage survival is unusually low, the percent of Habrobracon which survived in each module is nearly the same, although the number of wasps per module varies between 30 and 50. The low overall survival in this experiment was most likely a result of the fact that the males used were not freshly eclosed.

Table 2 presents the results of the study in which only control modules were used. No difference in survival is evident over the range from 12-30 Habrobracon per module layer. There is no indication of a differential survival between the Habrobracon in the middle layer and the Habrobracon in the two outer layers. In all of the module layers the percent survival is greater than 93, with 100% survival in 16 of the 18 module layers.

Table 1

Survival of Habrobracon males (Raleigh) maintained for four days and two hours in Habrobracon radiation modules

No. Wasps per Module	Total No. Wasps	No. Wasps Surviving	% Survival
30	90	73	81.1
40	160	117	73.1
50	200	147	73.5

Table 2

Survival of Habrobracon males (Raleigh) maintained for four days in control modules

Module Position*	Layer†	No. Wasps	No. Survivors	% Survival
	inner	15	15	100
1 Upper left	middle	15	14	93.3
	outer	15	15	100
	inner	20	20	100
1 Lower right	middle	20	20	100
	outer	20	20	100
	inner	25	25	100
1 Upper right	middle	25	25	100
	outer	25	25	100
	inner	30	30	100
1 Lower left	middle	30	30	100
	outer	30	30	100
	inner	20	20	100
3 Upper left	middle	20	20	100
	outer	20	19	95
	inner	12	12	100
3 Upper right	middle	12	12	100
	outer	12	12	100

*Position of the module in a bracket.

†Inner, middle, and outer refer to the 3 layers in a Habrobracon control module.

Discussion

The results of this study indicate that 20 Habrobracon per module is not close to the point at which Habrobracon would die as a result of overcrowding. Survival in the radiation modules was not significantly affected by increasing the number of Habrobracon in the module from 30-50. However, as already indicated in the previous section, the overall survival in this experiment was low because the Habrobracon were 6-8 days old when the study was initiated.

Survival was very high in the experiment in which the control modules were used. The males used in this experiment were freshly eclosed, no male being more than 8 hr old at the time the experiment was initiated. Therefore, it seems that similar males should be used at the time of launch.

The results of this study do not indicate that the number of Habrobracon which survive in the middle layer of the control module is lower than the number of Habrobracon which survive in the two outer layers of the module. It is possible that older Habrobracon that are placed in the middle layer are less viable than freshly eclosed Habrobracon, as the middle layer of the control module is probably compressed by the two layers of Habrobracon on either side. In any case, the overall survival of freshly eclosed Habrobracon in the control modules is high enough so that a slight depression in the survival of the middle layer of the control module is of little consequence.

Summary

1. The purpose of the study was to determine the number of Habrobracon which could be placed in each module or module layer for a 4-day period without killing the Habrobracon by overcrowding, and to also determine whether there is a difference in survival after 4 days between the middle layer of the control modules and the outer layers of the control modules.

2. No difference was observed in the percent survival in control module layers and modules over a range from 12 Habrobracon per module layer to 30 per module layer, and 30 Habrobracon per module to 50 per module.

3. There was no difference between the number of Habrobracon which survived in the middle layers of the control modules and the two outer layers of the control modules.

4. The overall survival of freshly eclosed, unfed males is much greater than 6-8 day old males which have been honey-fed.

Literature Cited

Smith, R. H., R. C. von Borstel, R. L. Amy, L. R. Valcovic, M. B. Baird, K. T. Cain, D. J. Goins, M. L. Haye, M. S. Jones, and E. S. Sirota, 1967, Biocompatibility Tests in Flight Vehicles, ORNL-TM-1732, Quarterly Progress Report to NASA covering the period July 1-Sept. 30, 1966, pp. 25-27.

IV. MEASUREMENT OF POSITIONS OF HABROBRACON PACKAGES IN THE BIOSATELLITE

R. C. von Borstel
Roger H. Smith
L. R. Valcovic
M. A. Daniele[1]
E. R. Park[2]
J. W. Gillespie[1]

In order to be able to repeat with precision the flight experiments in the ground controls, exact measurements were made of the positions of the Habrobracon packages. The actual locations of all the Habrobracon packages were measured in the flight vehicle with the packages in place. The packages were shifted as far as possible both toward the center and toward the periphery of the backscatter shield. The nominal 500-R package was twisted clockwise and counterclockwise, and the best positions for most uniform irradiation of the packages were adjudged.

Results and Conclusions

Two measurements were made of each package: minimum distance from the source when loosened, and maximum distance. The results are shown in Table 1. It appears

Table 1

Measurements of package positions on the backscatter shield

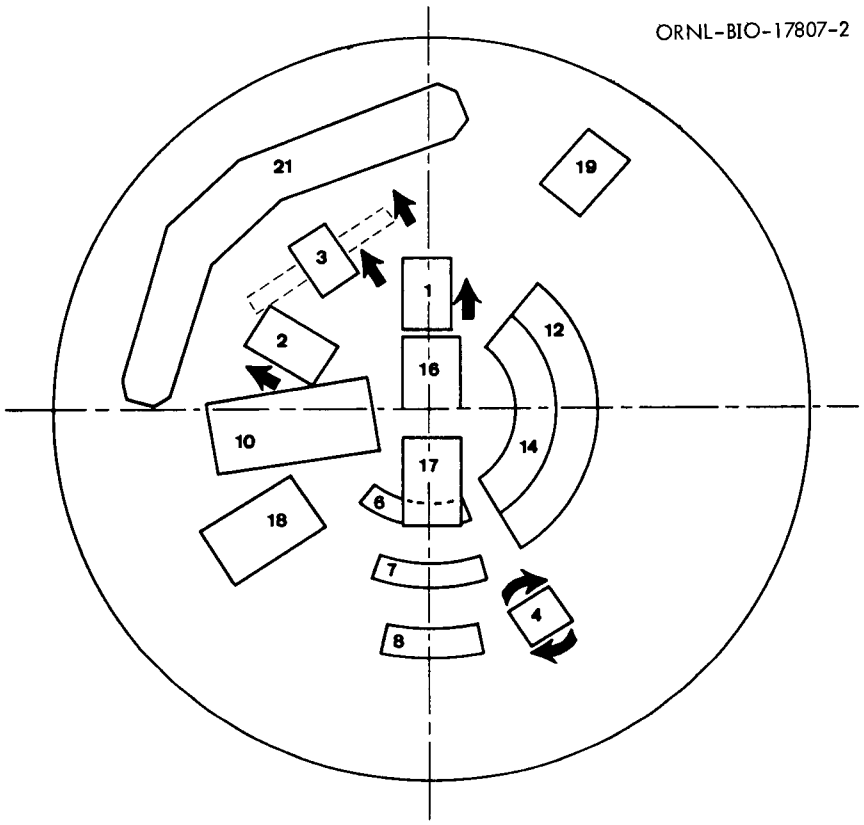
Package Number	Nominal Exposure (R) Position	Minimum Distance from Source (inches)	Maximum Distance from Source (inches)
A822	4000	2.567	2.602
A820	2000	3.833	3.853
A821	1000	5.351	5.466
A823	500	8.251	8.343

[1]Reentry Systems, General Electric, Philadelphia, Pa.

[2]Biosatellite Project, Ames Research Center, Moffett Field, Calif.

that some, but little, play is present in the bolt holes on the backscatter shield. It was concluded that, in order to minimize variation and to optimize repeatability, packages A822 and A820 would be pulled full away from the source before tightening in place, that both the bracket holding package A821 and the package itself would be pulled full away from the source before tightening, and that package A823 would be cocked as far away from the shield on its left as possible before tightening (see Fig. 1).

These conditions were written into the Flight-Time Instructions.



(1) Habrobracon A822
(2) Habrobracon A820
(3) Habrobracon A821
(4) Habrobracon A823
(6) Lysogenic Bacteria A828
(7) Lysogenic Bacteria A829
(8) Lysogenic Bacteria A827
(10) Tribolium A815
(12) Drosophila, Larvae A826
(14) Drosophila, Adult A825
(16) Neurospora A819
(17) Neurospora A817
(18) Neurospora A818
(19) Neurospora A816
(21) Tradescantia A824

FORWARD PAYLOAD EXPERIMENT PLATE LAYOUT

V. MEASUREMENT OF THE POSITIONS OF THE HABROBRACON NOMINAL 500-R PACKAGE IN THE BIOSATELLITE AND IN THE GROUND-BASED CONTROL PRIOR TO LAUNCH

R. C. von Borstel

M. A. Daniele[1]
E. R. Park[2]

On the morning of 14 December 1966, the Habrobracon packages were placed on the backscatter shield of the flight vehicle according to the Flight-Time Instructions. The instructions are to move the Habrobracon packages as far as possible away from the ^{85}Sr source before tightening them on the backscatter shield. The nominal 500-R Habrobracon package is to be swung away from the bracket beside it as far as it will go before tightening it in place. This is to reduce the shadowing of the nominal 500-R package by the new lysogenic bacteria package between the Habrobracon package and the source (see Fig. 1).

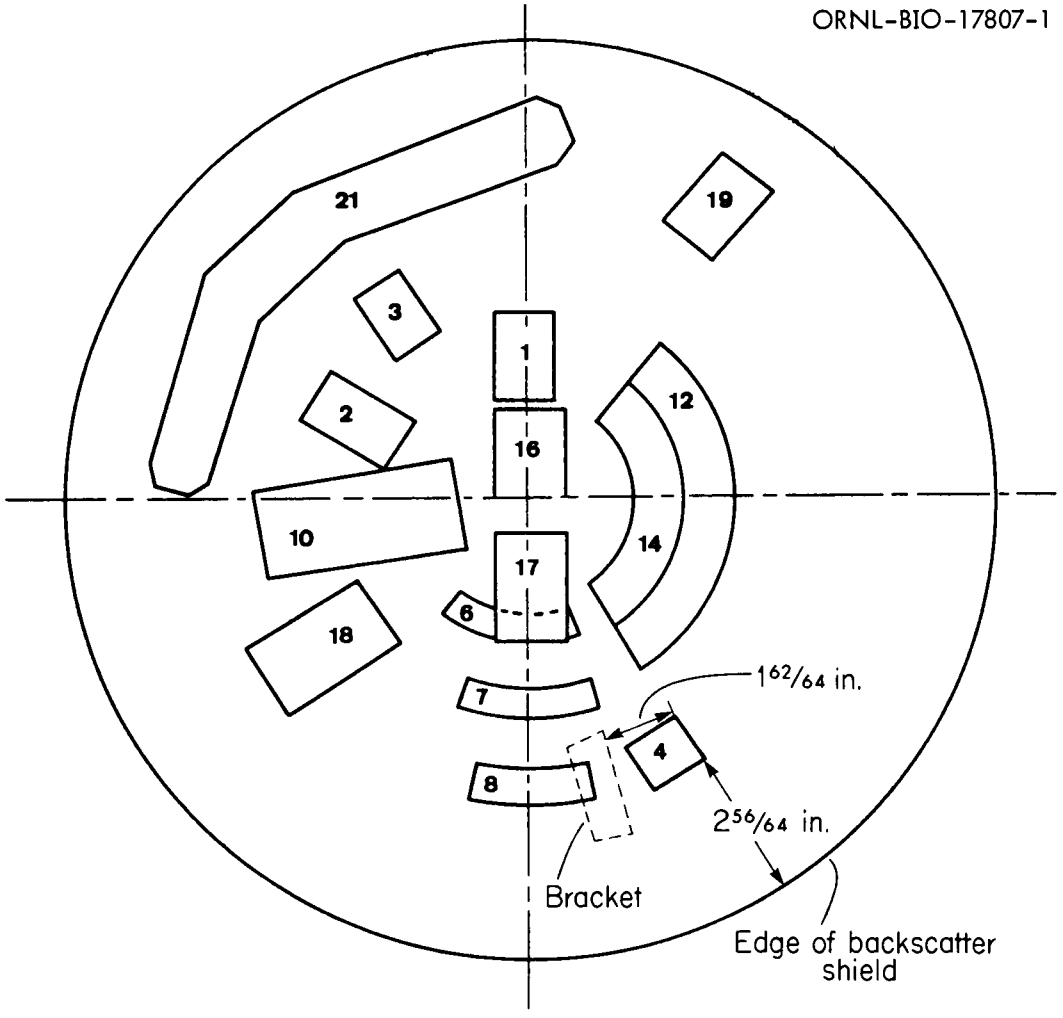
A measurement was then taken from the edge of the metal support nearest the nominal 500-R Habrobracon package to the further edge of the electron equilibrium shield on the package. This distance was 1-62/64 inches. A measurement from the rear of the package to the edge of the backscatter shield was also taken; this was 2-56/64 inches. Immediately afterward, similar measurements were made on the ground-based control. The distances were identical. The two edges toward the source had a full 1/2 inch over which they could be moved in relation to one another on the backscatter shield. The assemblies were put together according to the same instructions.

It appears that the best possible arrangement has been made for controlling the positions of the packages.

---

[1]Reentry Systems, General Electric, Philadelphia, Pa.

[2]Biosatellite Project, Ames Research Center, Moffett Field, Calif.



(1) Habrobracon A822
(2) Habrobracon A820
(3) Habrobracon A821
(4) Habrobracon A823
(6) Lysogenic Bacteria A828
(7) Lysogenic Bacteria A829
(8) Lysogenic Bacteria A827
(10) Tribolium A815
(12) Drosophila, Larvae A826
(14) Drosophila, Adult A825
(16) Neurospora A819
(17) Neurospora A817
(18) Neurospora A818
(19) Neurospora A816
(21) Tradescantia A824

FORWARD PAYLOAD EXPERIMENT PLATE LAYOUT

VI. GODDARD SPACE FLIGHT CENTER OPERATIONS FOR BIOSATELLITE A

R. L. Amy

On 13 December, approximately 24 hr before T-0, direct-line telephone communications were established with the laboratory control center at Cape Kennedy and with the recovery laboratory at Hickam Field. These links were exercised on a regular basis throughout the remainder of the mission. From 1200 hr on 14 December (launch occurred at 1420 E.S.T.) until the termination of the flight on 19 December, at least two of the three biologists involved (Rudy Hoffman, Ed Keller, and I) were on duty manning the two assigned control center consoles.

One of our main duties involved the acquisition, evaluation, and reporting of orbital telemetry data from the spacecraft. Data on each orbit were received by one of the STADAN stations; then they were relayed to the control center at GSFC. These were made available to us as computer print-outs (see Fig. 1 which is one of the actual print-outs for orbit 29). We immediately recorded on the Check List (Value column, Fig. 2) certain data from the print-out (A1, A2 . . . A115) adjudged most relevant to the biological aspects of the flight; thermistor temperatures were recorded on the Orbital Temperature form in the Flight column of Fig. 3. If any of the parameters fell outside the specified ranges, their interpretation was discussed with appropriate subsystem specialists or control room personnel. The data were then transmitted by voice to laboratory control at Cape Kennedy and to the Hickam Field laboratory. At the same time temperatures for control groups 1 and 2, being maintained at Cape Kennedy, were received and recorded in the proper columns (no. 1 and 2, Fig. 3). We calculated temperatures for fore and aft no. 3 controls, using selected thermistor readings from appropriate orbits, and transmitted them to both laboratories.

We were concerned also with checking the telemetry data to ascertain whether or not certain commands, of special significance to the biological experiments, had been received and executed by the spacecraft control systems. According to information received at GSFC, the following events occurred: (1) The radiation source opened on schedule at T+59 min and closed as planned on orbit 44. (2) The camera in the pepper plant package was functioning although it was not possible to verify that it was following its programmed schedule. (3) Fixing programs for frog eggs and wheat seedlings were verified as having occurred on schedule. However, the fixing-feeding schedule for amoebae apparently was advanced so that event number one occurred at T-1 min instead of at T+59 min. All subsequent programmed events for the amoeba package then apparently occurred one sequence earlier than planned.

On the 47th orbit, when it became obvious that a malfunction in the deorbiting sequence had occurred and the recovery capsule would not return on schedule, our activities were terminated.

Figure 1. Spacecraft telemetry data print-out. (Biosatellite A, orbit 29)

```
                                   BIOSATELLITE      REAL TIME                    VEHICLE 302

     FRAME  122              GMT  350  14  54  33                ORBIT NO. 29F   STATION      FT. MYERS

      ATTITUDE CONTROL SUBSYSTEM                          LIFE SUPPORT SUBSYSTEM                ELECTRICAL POWER SUBSYSTEM

A1   R RATE  PRIMARY 30  .106 DES      A50   REC CAP TEMP      25  62.5 DEF          +28 VDC REG AD   35  28.0 VDC
A2   P RATE  PRIMARY 31 -.020 DES      A51   REC CAP REL HUM   33  52.5 PCT          -28 VDC REG AD   39 -28.1 VDC
A3   Y RATE  PRIMARY 34 -.110 DES      A52   REC CAP T PRESS   48  15.3 PSA          +10 VDC REG AD   45  10.0 VDC
                                       A53   REC CAP PART 02   19  150. MMH          -10 VDC REG AD   22 -10.0 VDC
     R RATE  SECOND  32  .059 DES                                                      +5 VDC REG AD   55  4.94 VDC
     P RATE  SECOND  32 -.054 DES                                                      +6 VDC REG AD   55  6.00 VDC
     Y RATE  SECOND  32 -.028 DES      A55   BLOWER  CURRENT   39  .131 AMP           -6 VDC REG AD   40 -6.09 VDC
                                       A56   RES AIR TK PRESS  57  3440 PSG
     C ROLL  IR SENS 37  4.40 DEG                                                     ORBTL BUSS FINE  30  27.4 VDC
     C PITCH IR SENS 37 -4.65 DEG                                                      26 VAC REG AD   49  25.7 VAC
     F PITCH IR SENS 02 -5.47 DEG                                                     OR BATT VOLTAGE  50  27.6 VDC
     F ROLL  IR SENS 02  4.87 DEG                                                A109 OR BATT CURRENT  26  4.10 AMP
     MAGNET  OUTPUT  21  10.0 MLG                                                A110 SIGNAL  GROUND   00  .000 VDC
                                          TANKAGE SUBSYSTEM
     R EARTH PRESNCE 01  0                                                            +10 VDC REG CAP  29  9.91 VDC LO
     P EARTH PRESNCE 01  0             N2 TANK TEMP      30  47.7 DEF                 -5 VDC REG CAP  33 -4.91 VDC
     YAW SIG SWITCH      0             A71 N2 TANK PRESS 49  2333 PSI              A115 +5 VDC REG CAP  50  5.00 VDC
                                                                                      115 VAC REG CAP  44  114. VAC
     TEMP R  IR SENS 20  38.1 DEF          ENVIRON. CONTROL SUBSYSTEM                  4.25VDC REG CAP  54  4.12 VDC
     TEMP P  IR SENS 21  40.2 DEF                                                     -2.5VDC REG CAP  36 -2.50 VDC
                                          OR BATT TEMP      25  70.0 DEF
     PRI-SEC GYRO    29  P                OR HEAT TEMP 1    26  64.0 DEF
     JET CON PWR ON      1                OR HEAT TEMP 2    25  62.7 DEF
     MAGNET  BOOM DE     1                OR HEAT TEMP 3    23  60.1 DEF
                                          OR HEAT TEMP 4    24  61.8 DEF
     POS R   SOLNOID     0                                                             DEORBIT SUBSYSTEM
     NEG R   SOLNOID     0                ADAPTER TEMP 1    25  44.7 DEF
     POS P   SOLNOID     0                ADAPTER TEMP 2    22  42.3 DEF              O W S P MONITOR  .000000  SEC
     NEG P   SOLNOID     0                ADAPTER TEMP 3    22  40.9 DEF                      0000000000000000
     POS Y   SOLNOID     0                ADAPTER TEMP 4    39  84.5 DEF              MAGNET  PROGRAM 00000000
     NEG Y   SOLNOID     0
                                          CAP Z1  TEMP      27  43.3 DEF LO A89        SEPARATE SENSOR  47  1
     N2 REG  PRESS   52  42.3 PSG HI      CAP Z2  TEMP      25  70.7 DEF A90        ACC-REJ COMMAND  63  1
                                          AFT COV TEMP RV   33  57.6 DEF LO           O W S P ERROR        0
                                                                                      VER ARM COMMAND      0
      RECOVERY SUBSYSTEM                                                              PROGRAM TIMER        43 30
                                                                                      TIMER CH 1       40
     BAROSW  MONITOR 38  1                                                            TIMER CH 2       43
     RECOVER BATT 1  00  .000 VDC                                                     TIMER CH 3       32
     RECOVER BATT 2  00  .000 VDC                                                     TIMER CH 4       53
```

BIOSATELLITE REAL TIME VEHICLE 302

FRAME 122 GMT 350 14 54 32 ORBIT NO. 29F STATION FT. MYERS

EXPERIMENT TEMPERATURES

THA151	P 1017	31	68.5	DEF	
THA152	P 1017	31	68.5	DEF	
THA153	P 1017	31	68.5	DEF	
THA154	P 1017	31	68.5	DEF	
THA156	P 1020	20	66.6	DEF	
THA157	P 1020	20	66.6	DEF	
THA158	P 1020	19	65.8	DEF	
THA159	P 1020	19	65.8	DEF	
THA160	P 1035	29	66.7	DEF	
THA161	P 1035	30	67.6	DEF	
THA162	P 1035	30	67.6	DEF	
THA163	P 1035	30	67.6	DEF	
THA164	P 1037	58	98.1	DEF	HI
THA165	P 1037	63	105.	DEF	HI
THA166	P 1037	46	83.7	DEF	HI
THA167	P 1037	32	69.5	DEF	
THA168	P 1037	32	69.5	DEF	
THA169	P 1039	48	85.8	DEF	
THA170	P 1039	48	85.8	DEF	

EXPERIMENT TEMPERATURES

THA171	P 1047	29	66.7	DEF	
THA172	P 1047	29	66.7	DEF	
THA173	P 1047	29	66.7	DEF	
THA174	P 1047	29	66.7	DEF	
THA179	P 1079	29	66.7	DEF	
THA180	P 1079	32	69.5	DEF	
THA181	P 1079	32	69.5	DEF	
THA182	P 1079	31	68.5	DEF	
THA183	P 1079	30	67.6	DEF	
THA184	P 1123	27	64.9	DEF	LB
THA185	P 1123	31	68.5	DEF	
THA186	P 1135	28	65.8	DEF	
THA187	P 1135	30	67.6	DEF	
THA188	P 1135	31	68.5	DEF	
THA189	P 1135	31	68.5	DEF	
THA190	P 1159	28	65.8	DEF	
THA191	P 1159	30	67.6	DEF	
THA192	P 1160	28	65.8	DEF	
THA193	P 1160	30	67.6	DEF	

EXPERIMENT EVENTS

A200	P 1020	55	0	SW/CL
A201	P 1035	03	1,2,3	SW/CL
A202	P 1035	36	1,2	SW/CL
A203	P 1035	50	1	SW/CL
A204	P 1035	36	1,2	SW/CL
A205	P 1035	03	1,2,3	SW/CL
A206	P 1035	03	1,2,3	SW/CL
A207	P 1035	03	1,2,3	SW/CL
A208	P 1035	03	1,2,3	SW/CL
A209	P 1035	03	1,2,3	SW/CL
A210	P 1035	36	1,2	SW/CL
A211	1035/47	56	0	SW/CL
A212	P 1047	03	1,2,3	SW/CL
A213	P 1047	03	1,2,3	SW/CL
A148	TAPE	OFF		
A150	SOURCE	OPEN		
A155	CAMERA	CLSE		

Fig. 1. Spacecraft Telemetry Data Print-out (Biosatellite A, orbit 29)

CHECK LIST

ORBIT # 29

STATION H Myers

GMT 350 14 54 05

Measurement #	Designation	Biol. Problem Limits	Value	Status	Comment
A1	Roll	.20°/sec	.150		
A2	Pitch	.20°/sec	.023		
A3	Yaw	.20°/sec	-.110		
A50	Incentive Temp.	75° to 65°F	62.5		
A51	R.H.	40 to 70 RH	52.5		
A52	Total Press.	13.2 to 16.2	15.3		
A53	ppO_2	139 to 169 mm Hg	150		
A55	Blower Curr.		.131		
A56	Air Tank P.		3440		
A71	N_2 Tank P.		2333		
A89	Capsule T. (1)	75 to 65° F	43.3		
A90	Capsule T. (2)	75 to 65° F	70.7		
A109	Orbit Batt.	Voltage 28	27.6		
A110	Orbit Batt.	Amperage	3.8		
A115	+5 VDC		4.12		

Fig. 2

BIOSATELLITE ORBITAL TEMPERATURES

TIME 350 1454 05 1452 Z ORBIT NUMBER 29

EXPERIMENT	DOSE	A NO	FLIGHT	NO. 1 CONTROL FLIGHT SIMULATION	NO. 2 CONTROL CONSTANT TEMPERATURE	NO. 3 CONTROL VARIABLE TEMPERATURE	COMMENTS
P-1017 PEPPER	NONE	151	69	72	71	67	
	NONE	152	69	72	71	67	
	NONE	153	69	72	71	67	
	NONE	154	69	73	71	67	
P-1020 WHEAT	NONE	156	66	71	72	66	
	NONE	157	67	70	72	65	
	NONE	158	66	70	72	66	
	NONE	159	66	70	—	66	
P-1035 AMOEBA	NONE	160	67	71			
	NONE	161	68	71			
	NONE	162	68	71			
	NONE	163	68	71			
					71		
P-1037 NEUROSPORA	NONE	164	100	71			
	500	165	105	70			
	1000	167	70	70			
	2500	166	85	70			
	6000	168	70	70			
					71		
P-1039 TRIBOLIUM	NONE	169	86	85			
	1300	170	87	86			
P-1047 FROG EGG	NONE	171	67	71			
	NONE	172	67	71			
	NONE	173	67	71			
	NONE	174	67	71			
					71		
P-1079 HABROBRACON	NONE	179	67	71			
	500	183	68	70			
	1000	181	70	70			
	2000	180	70	70			
	4000	182	69	70			
					71		
P-1123 TRADESCANTIA	NONE	184	65	70			
	300	185	69	70			
P-1135 BACTERIA	NONE	186	66	—			
	500	187	68	—			
	1000	189	69	70			
	2500	188	69	—			
P-1159 ADULT DROSOPHILA	NONE	190	66	70			
	2000	191	68	69			
					71		
P-1160 LARVA DROSOPHILA	NONE	192	66	70			
	1300	193	68	70			
					71		

ARC 536 (NOV 66)

Fig. 3

VII. THE FLIGHT OF BIOSATELLITE A

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Biosatellite A was launched at 1420 hr on 14 December 1966. All phases of the Habrobracon experiment proceeded smoothly and were either slightly ahead of or on schedule.

The Loading of the Modules

By careful scheduling over 43 days prior to launch, it was possible to obtain enough Habrobracon males that had eclosed within 24 hr of the loading. The females had all eclosed within 3-5 days prior to launch and their pedigrees were established.

The actual numbers of Habrobracon males and females loaded into the modules are shown in Table 1. The flight spares were used as additional controls in the Control II and Control III series when they were no longer needed as back-up packages for loading the vehicle.

Table 1

Numbers of Habrobracon males and females loaded into flight and ground-based control packages for Biosatellite A

	Control Area		Radiation Area		Subtotals	
	♂	♀	♂	♀	♂	♀
Flight	120	90	160	120	280	210
Flight spares	120	90	80	60	200	150
Control I	120	90	160	120	280	210
Control II	200	120			200	120
Control III	200	120			200	120
Total					1160	810

In addition to these animals, 1200 young Habrobracon females with an established pedigree for each were available to take to Hickam Field for mating with the males that were to be returned from the flight.

Temperature Data from the Biosatellite

The only records unique to the Habrobracon experiment were the orbital thermistor readings which provided data on the temperature of the air between the modules on each package. These and similar measurements from Controls I and II are listed in Table 2 (next page). It is apparent that Control I, the most important control, was consistently higher by 3°F in the aft, low-exposure area, and was often within a degree of the temperature in the fore, radiation area, of the spacecraft.

Difficulty at Time of Reentry

We were prepared both at Cape Kennedy and Hickam Field for receipt of the Habrobracon packages. Unfortunately, the rocket engine (Fig. 1), which was to slow the vehicle for reentry into the atmosphere, failed to function. After failure of several attempts to bring the craft down on successive orbits, the ground-based controls at Cape Kennedy were released to the investigators. The analyses were pursued on these.

ORNL-BIO-18377

Fig. 1. The Retro-Rocket of Biosatellite A.

Table 2

Biosatellite orbital temperatures for the Habrobracon experiment

Date	Time	Orbit	Flight Temperature					Control I Temperature					Control II Temperature
			0	500	1000	2000	4000	0	500	1000	2000	4000	
12/14	1340	Pre-launch	68	70	70	71	70						
	1424	Pre-launch	66	71	71	71	71						
	1452	Pre-launch	66	71	71	71	71						
	1519	Pre-launch	65	69	71	71	70						
	1600	Pre-launch	66	71	71	71	71						
	1633	Pre-launch	66	71	71	71	70						
	1700	Pre-launch	66	70	71	71	70						
	1814	Pre-launch	67	70	70	70	69						
	1816	Pre-launch	68	70	71	71	71						
	1900:55	Pre-launch	68	70	71	71	71						
	2020	Launch											
	2100	1	68	69	70	71	70	73	75	75	75	74	70
	2237:17	2	68	68	69	70	69	73	73	73	73	73	70
12/15	0016:54	3	68	67	69	70	68	70	69	70	70	70	71
	0153:40	4	67	67	69	69	68	70	68	69	69	69	71
	0331:02	5	67	67	69	69	68	69	68	68	68	68	71
	0507	6	67	67	68	69	68	67	68	67	67	68	70
	0643:23	7	67	67	68	69	68	72	73	73	73	72	71
	0819:46	8	67	67	68	69	68	71	71	71	71	71	71
	0954:30	9	67	67	68	69	68	71	70	70	71	71	71
		10	67	67	68	69	68	71	70	70	71	71	71
	1143:42	11	67	67	68	69	68	71	70	70	71	71	71
	1311	12	67	67	68	69	68	70	69	70	70	72	71
	1445:46	13	67	67	68	69	68	70	68	69	69	69	
	1619:31	14	67	69	69	68	68	70	69	69	69	69	72
	1759:53	15	67	68	69	70	69	68	67	67	68	68	72
	1931:48	16	67	68	69	69	68	70	69	69	70	69	71
	2107:14	17	67	68	69	70	69	71	70	70	71	70	72
	2248:02	18	68	68	69	70	69	70	70	70	70	70	72
12/16	0029:12	19	67	68	69	69	69	70	70	70	70	70	72
	0205:01	20	67	68	69	69	69	70	70	70	70	70	72
	0339:35	21	68	68	69	70	69	70	70	70	70	70	67
	0516:01	22	68	68	69	70	69	70	70	70	70	70	70
	0650	23						70	70	70	70	70	70
	0828	24	68	68	70	70	69	71	70	70	70	70	70
	1007:20	25/26	68	68	69	70	69	70	70	70	70	70	70
	1142:36	27	68	68	70	70	69	70	70	70	70	70	70
	1316:22	28	68	68	70	70	69	71	70	70	70	70	70
	1454:05	29	67	68	70	70	69	71	70	70	70	70	71
	1632:26	30	67	69	70	70	69	70	68	69	69	69	71
	1807:35	31	67	68	69	70	69	69	68	68	68	68	68
	1941:20	32	67	68	70	70	69	71	71	71	71	71	70
	2120:42	33	68	68	69	70	69	71	70	70	70	70	69
	2257:30	34	68	70	70	69	68						
12/17	0037:45	35	67	68	69	70	69	71	70	70	70	70	69
	0212:41	36	67	68	69	70	69	71	70	70	70	70	71
	0348:10	37	67	69	70	70	69	71	70	70	70	70	70
	0524:00	38	67	69	70	70	69	71	70	70	70	70	70
	0701:40	39	67	69	70	70	69	71	70	70	70	70	70
	0837:33	40	67	69	70	70	69	71	70	70	70	70	70
	1017:01	41/42	67	70	70	69	69	70	69	69	69	69	70
	1151:28	43	68	69	70	70	69	70	68	68	69	69	70
	1324	44	67	69	70	70	69	70	68	68	69	69	70
	1506:09	45	67	69	70	70	69	69	68	68	68	68	70
	1642:23	46	67	69	70	70	69	72	72	72	72	72	70

VIII. EVALUATION OF FLIGHT-TIME PREPARATIONS FOR THE HABROBRACON (P-1079) EXPERIMENT IN THE FIRST BIOSATELLITE

R. C. von Borstel Roger H. Smith

During the preparations over the years prior to the flight we knew that it would be essential to have approximately 10-15 people available for the Biosatellite Experiment. This is necessary because one investigator can effectively perform all of the operations on only about 200 Habrobracon by himself, and only then by maintaining a working day of 10-12 hr and a working week of 6 or 7 days. Habrobracon are relatively short-lived, but a large amount of information can be gained from each one. Therefore, in order to obtain meaningful amounts of data in a single large experiment such as the Biosatellite, a number of competent investigators are required. Normally, in order to accumulate significant numbers of data, the same experiment is repeated several times. It is not possible to do this with the Biosatellite experiment.

It is clear that the various rehearsals and walk-thrus at Cape Kennedy prior to the launch were an exceedingly valuable preparation. The work flowed automatically and smoothly; it is doubtful if this would have been the case without the walk-thrus.

Personnel

In the first walk-thru, six investigators were used in dealing with flight preparations at Cape Kennedy. It was obvious that this was a minimal number, and for the remaining walk-thrus and the flight itself, eight investigators took part in the activities at Cape Kennedy.

Two investigators took care of culturing the wasps at Cape Kennedy which were used for the flight of the Biosatellite. The bare minimum of males and virgin females

was obtained by launch day, but efficiency and yield of wasps would have been better with more investigators working at Cape Kennedy.

For the next Biosatellite experiment, four investigators will be responsible for wasp cultures in order to increase the efficiency of handling glassware and host cultures, record keeping, and collection of virgin females. This would assure a yield of wasps above the minimum necessary for the experiment.

After the flight, three people went to Hickam Field for the work associated with recovery of the Biosatellite, two went to Oak Ridge to prepare for the large influx of experimental material, and three stayed at Cape Kennedy to tend the controls on 8-hr shifts and to carry out the necessary operations associated with the controls at the time of recovery.

It was found that the three people at Cape Kennedy were not sufficient to successfully carry out all the demanding post-flight operations. This was not possible for these reasons:

(1) The 8-hr shifts for 3 days were extremely taxing to the lone investigators on each shift, particularly those working at night. With myriad duties better carried out in concert, it was a trying experience to keep a continuity of work getting ready for the post-flight operations.

(2) Since the shift work lasted for only 3 days, it was not possible for the investigators on the night shift to get enough rest for the monumental task associated with post-flight operations. One investigator went for 40 hr without sleep when post-flight operations began, slept for 3 hr, and then worked 24 more. His efficiency was impaired.

(3) We knew that the number of personnel was minimal at the Cape and at Hickam Field, but we underestimated the time that would be required for making observed matings of all the Habrobracon; consequently only one-half of all the Habrobracon used were controlled by observed matings. Fortunately, about one-half the remainder mated, making the ground-based control an extremely successful experiment. This could have ended disastrously.

Therefore, for the Habrobracon post-flight operations at the second launch, it will be necessary to increase the number of investigators at both Hickam Field and Cape Kennedy in order not to jeopardize the experiment. We shall require six investigators at each place and shall cut back on the number of investigators at Oak Ridge. The Oak Ridge cutback can be achieved by preparing for most of the Oak Ridge operations before the flight. Only the collection of Ephestia larvae for this experiment will be impaired by this cutback, and some arrangement will be made for this by the time of the flight.

The Habrobracon

The Raleigh wild-type strain of Habrobracon used in the Biosatellite experiment is a hardy strain with high hatchability and high adult survival. It has the disadvantage that the females do not mate willingly after they are over a week old. Many strains of Habrobracon are like this, but this is particularly serious since, with the many operations associated with the flight, all of the females must be collected as virgins during the 2- to 5-day period preceding the flight. With the flight lasting 3 days and the immediate post-flight operations another 1-4 days, depending on the circumstance, the females are usually at least a week old before mating begins.

The collection of virgin females was achieved satisfactorily at the time of the Biosatellite Flight on 14 December 1966. By an elaborate scheme of pedigree analysis, we were able to avoid pretesting the females which we ordinarily do to select against the females in the collection which were heterozygous for recessive lethals. This pedigree analysis was successful, and a program for it is being written for the computer.

We shall have an alternate strain of male Habrobracon ready for Biosatellite B. In the flight of Biosatellite A, the lemon mutant strain was used. Although it has been tested for flight hardiness and is quite satisfactory, a hardier strain, the Lumberton D wild-type, will be held in readiness as a possible last minute substitute.

IX. STANDING INSTRUCTIONS AND FLIGHT-TIME INSTRUCTIONS FOR THE HABROBRACON (P-1079) EXPERIMENT IN THE THREE-DAY BIOSATELLITE

Table of Contents

1 PRE-FLIGHT OPERATIONS IN OAK RIDGE

1.1 Rearing of host Ephestia

1.2 Collection of Ephestia larvae

1.3 Preparation of glassware and auxiliary equipment for Habrobracon culture

1.4 Cleansing and testing of modules

1.5 Rearing of Habrobracon

1.6 Testing of stock for spontaneous lethal mutations

2 PRE-FLIGHT OPERATIONS AT CAPE KENNEDY AND HICKAM FIELD

2.1 Equipment and biological material provided by experimenter

2.2 Equipment provided by NASA

2.3 Handling the Habrobracon and Ephestia

2.4 Cleansing of culture glassware and modules

2.5 Dosimetry preparation

2.6 Pre-flight preparation of Habrobracon

2.7 Preparation and assembly of the Biosatellite package

2.8 Calibration of equipment at Cape Kennedy

2.9 Calibration of equipment at Hickam Field

3 IN-FLIGHT OPERATIONS AT CAPE KENNEDY AND HICKAM FIELD

3.1 Labeling vials and Stender dish trays

3.2 Readout of environmental conditions and plans for emergencies

3.3 Preparation of score sheets

3.4 Preparation of females for mating

4 POST-FLIGHT OPERATIONS AT CAPE KENNEDY AND HICKAM FIELD

4.1 Package disassembly

4.2 Handling the Habrobracon

5 PERSONNEL ASSIGNMENTS

5.1 Deployment of personnel

5.2 Assembly teams

5.3 Deployment for shift duties during the flight

5.4 Disassembly teams

6 POST-FLIGHT OPERATIONS ON THE HABROBRACON EXPERIMENT

6.1 The Habrobracon experiment at Hickam Field, Cape Kennedy, and Raleigh

6.2 The Habrobracon experiment at Oak Ridge

1. Pre-Flight Operations in Oak Ridge

1.1 Rearing of Host Ephestia

1.1 Rearing of host Ephestia

1.1.1.0 Scope: This instruction comprises the equipment and technique for rearing the host Ephestia larvae to culture Habrobracon for stock and experimental use

1.1.2.0 Equipment

1.1.2.1 Wax paper bags 6 X 7 X 5/8 inches. Vendor: Dillard Paper Company, Knoxville, Tenn.

1.1.2.2 Culture (shirt) boxes, 9-3/4 X 14 X 2 inches. Vendor: Knoxville Paper Box Company, 200-206 E. Magnolia Avenue, Knoxville, Tenn.

1.1.2.3 Lucite boxes, 13-1/8 X 9 X 2-5/16 inches. Trans Box, P 801. Vendor: Vlchek Plastics Co., Middlefield, Ohio

1.1.2.4 Coarse Corn Meal: Yellow Hybrid Corn, Perry Lamb Feed Mill, Tusculum, Tenn.; Quaker Yellow Corn Meal (1 lb 8 oz boxes) (enriched and degerminated), Quaker Oats Co., Riverside Drive, Chattanooga, Tenn.

1.1.2.5 Shell vials (See 1.3.2.1.1)

1.1.2.6 Refrigerator 7-10°C

1.1.2.7 Warm-room 30°C (See 1.5.2.10)

1.1.2.8 Paper towels. Wendell Ceefold Towels. Vendor: Erving Paper Mills, Erving, Mass.

1.1.2.9 Plastic wash bottle. Distilled H_2O.

1.1.3.0 Techniques for collecting adult Ephestia for oviposition

1.1.3.1 Pour 100 g corn meal into a wax paper bag

1.1.3.2 Remove lid of Ephestia culture box in which adults are sitting

1.1.3.3 With a shell vial collect 40-50 adult Ephestia by gently touching their heads (Fig. 1.1-1)

1.1.3.4 The adult Ephestia are shaken into a wax paper bag containing corn meal (Fig. 1.1-2)

1.1.3.5 The wax paper bag is twisted shut

1.1.3.6 The paper bags are stored at room temperature or 30°C

1.1.3.7 In approximately 2 weeks small Ephestia larvae appear in the bags of corn meal (Fig. 1.1-3)

1.1.4.0 Technique for culturing Ephestia larvae

1.1.4.1 Spread approximately 500 g of corn meal on the bottom of the culture box (See 1.1.2.2 or 1.1.2.3)

1.1.4.2 The contents of a bag are mixed with the corn meal in the culture box

1.1.4.3 A moistened paper towel is placed over the corn meal and the culture-box cover is replaced

1.1.4.4 Culture boxes are kept at room temperature and checked three times a week. Corn meal and water are added when needed

1.1.4.5 When the Ephestia larvae are full grown the paper towels on top of the corn meal are moistened and culture boxes are put into a refrigerator at 7-10°C

Fig. 1.1-1. Collection of Adult Ephestia with a Shell Vial.

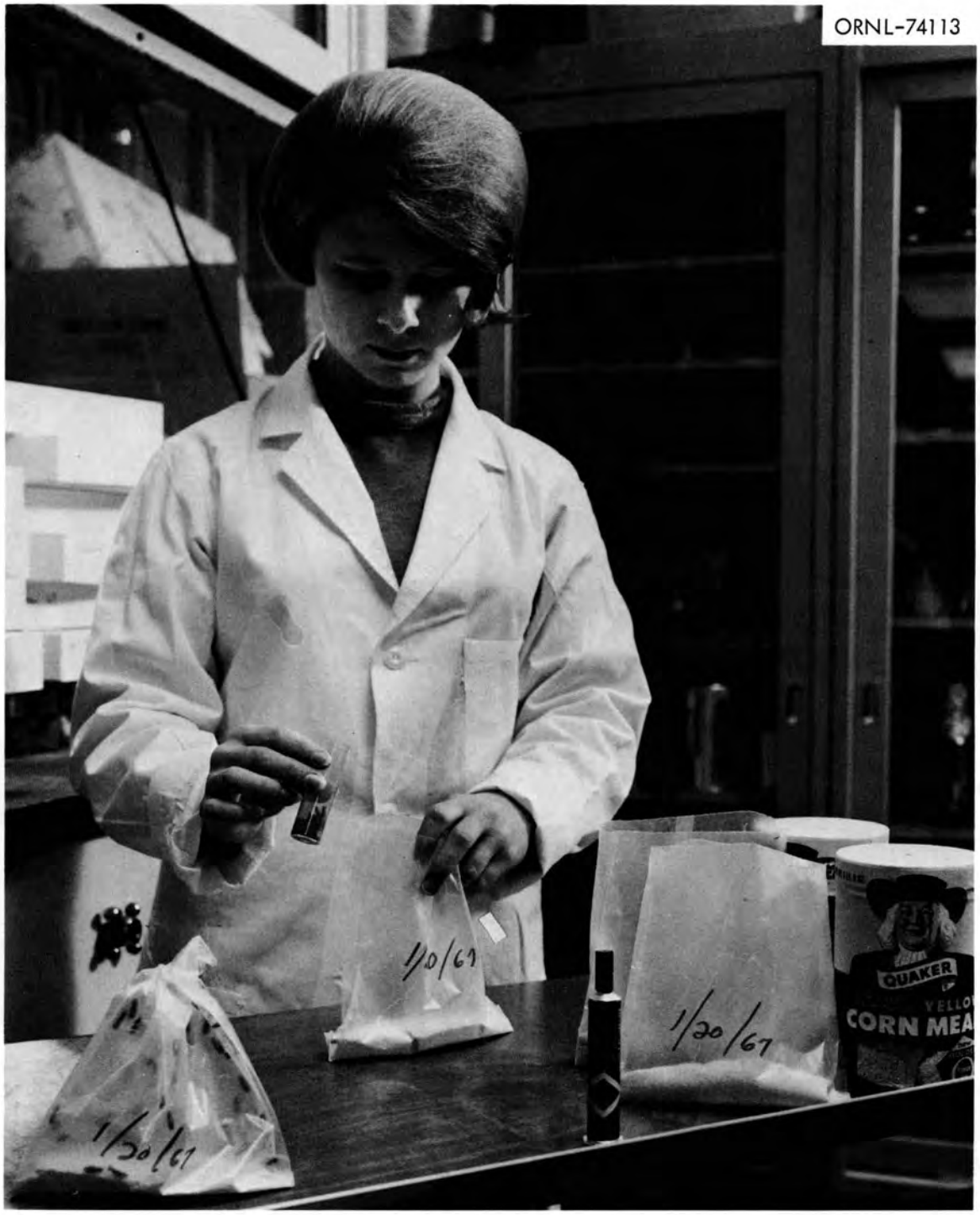


Fig. 1.1-2. Adult Ephestia Are Put Into a Wax Paper Bag Containing Corn Meal.

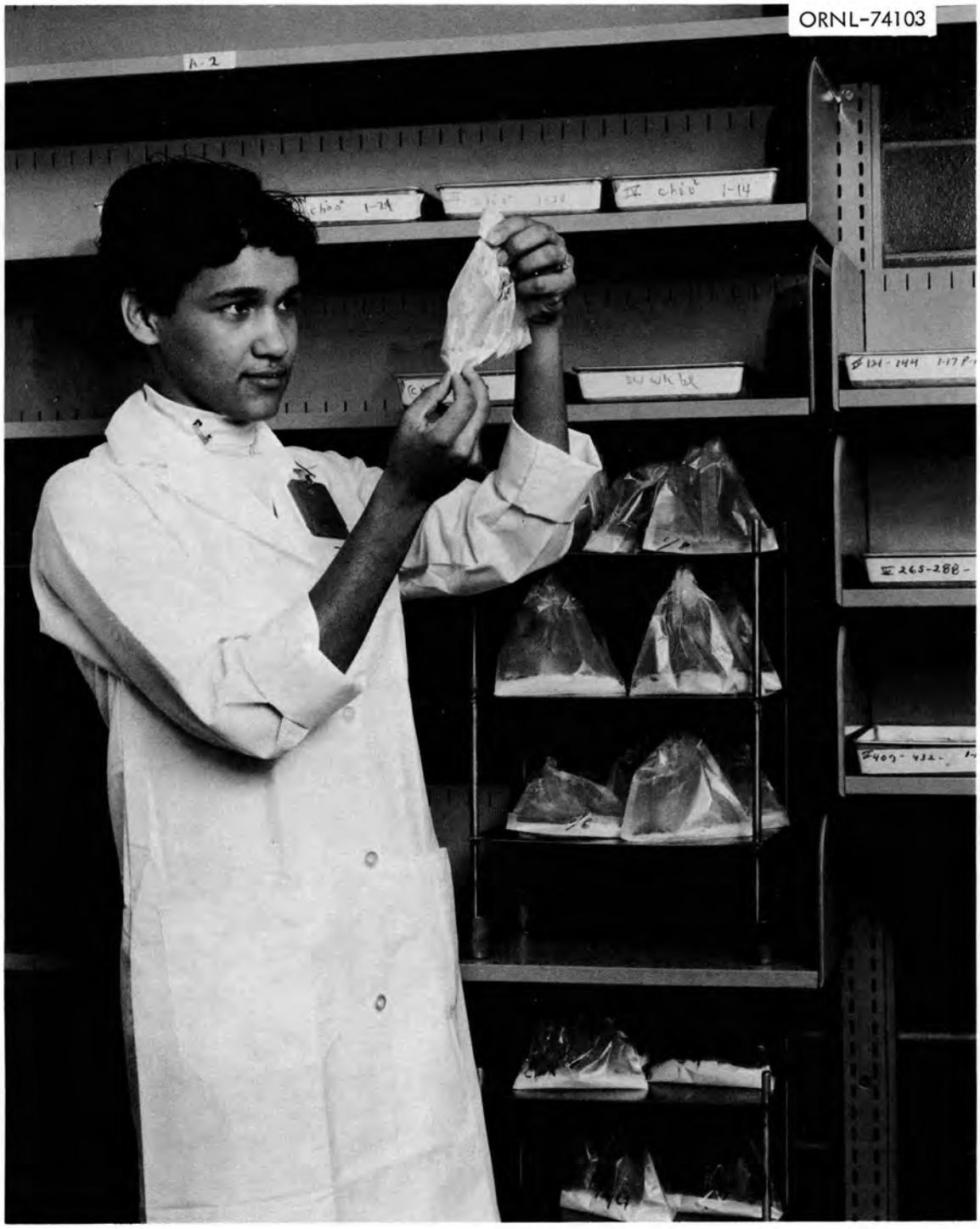


Fig. 1.1-3. Small Ephestia Larvae Can Be Seen by Examination of the Bags of Corn Meal.

1.2. Collection of Ephestia larvae

1.2. Collection of Ephestia larvae

1.2.1.0 This instruction comprises the technique in collecting Ephestia larvae

1.2.2.0 Equipment

1.2.2.1 Brass forceps

1.2.2.2 Ephestia larvae collection dishes

1.2.2.3 Refrigerator 7-10°C

1.2.2.4 Ephestia larvae culture boxes (See 1.1.4.5)

1.2.3.0 Collection and storing of Ephestia larvae

1.2.3.1 An Ephestia culture is taken from the refrigerator and the cover removed

1.2.3.2 The paper towel is turned back. Full grown Ephestia larvae are picked up gently with brass forceps and dropped into an Ephestia larvae collection dish (Fig. 1.2-1)

1.2.3.3 The maximum number of Ephestia larvae normally placed in a dish is 500

1.2.3.4 The Ephestia larvae are allowed to crawl about for 2-3 hr at room temperature until they become free of corn meal dust

1.2.3.5 The Ephestia larvae collection dish is then placed into the refrigerator until the larvae are to be used

1.2.3.6 The paper towels are moistened with distilled H_2O and the Ephestia culture boxes are placed back into the refrigerator

1.2.3.7 Ephestia culture boxes usually hold from 2000-4000 larvae; thus larvae can be collected from them a number of times over a 1- to 6-month period

1.2.3.8 When emptied of large larvae, and few more will be developing, 70% alcohol is poured over the surface and the boxes are discarded

Fig. 1.2-1. Full-Grown Ephestia Larvae Are Picked Up Gently with Brass Forceps and Dropped into an Ephestia Larvae Collection Dish.

1.3. Preparation of Glassware and Auxiliary Equipment for Habrobracon Culture

1.3. Preparation of glassware and auxiliary equipment for Habrobracon culture

1.3.1.0 Scope: This instruction covers the specifications for glassware, the specifications for cleansing and drying the glassware, the specifications for the auxiliary equipment, the placement of the shell vials and Stender dishes in baskets for washing, the technique by which the shell vials are etched, the storage of clean shell vials for use by experimenters, and the placement of Stender dishes in aluminum pans by the experimenters. The shell-vial stoppers, morgues, storage cartons, and trays are also described

1.3.2.0 Equipment

1.3.2.1.0 Glassware

1.3.2.1.1 Shell vials: 70 mm long X 21 mm diameter. Vendor: Arthur H. Thomas Co., Philadelphia, Pa.

1.3.2.1.2 Stender dishes: 37 X 19 X 24 mm with ground glass matching of dish and cover. Vendor: Arthur H. Thomas Co., Philadelphia, Pa.

1.3.2.2.0 Cleansing equipment

1.3.2.2.1 Dish washer: Better Built Turbomatic Model No. 3500, Better Built Machinery Corp., 73-75 East 130th Street, New York, N.Y. Tap water temperature 190°F; distilled water temperature 190°F

1.3.2.2.2 Baskets for shell vials: 6 X 6 X 6 inches, Model No. 666 B with covers. Model No. 66313 provided by Better Built Machinery Corp.

1.3.2.2.3 Trays for Stender dish covers: 30 X 30 X 1 inches. Also used for dishes

1.3.2.2.4 Drying oven: Hotpack Model No. 624, Hotpack, Philadelphia, Pa. Hot-air temperature 170°F

1.3.2.2.5 Bottle-washer brush: Model No. 300-B, Southern Cross Mfg. Corp., Chambersburg, Pa.

1.3.2.3.0 Auxiliary equipment

1.3.2.3.1 Etching fluid: Miracle Etching Cream. Vendor: Etchall Inc., Columbia, Mo.

1.3.2.3.2 Labels: Gummed Labels, No. B-120, Denny-Reyburn Co., West Chester, Pa.

1.3.2.3.3 Cotton: Nonsterile, blended 50% with Safron, nonsurgical absorbant. Vendor: Johnson & Johnson, New Brunswick, N.J.

1.3.2.3.4 Cloth: Cheesecloth, No. 120, 72 X 56 inches. The Kendall Co., Textile Div., 111 W. 40th Street, New York, N.Y.

1.3.2.3.5 Thread: Heavy-duty thread used for overcoats. Vendor: Clark

1.3.2.3.6 Aluminum pans: Dissecting pans, 7-1/2 X 11 X 1-1/2 inches. Vendor: General Biological Supply House, Chicago, Ill.

1.3.2.3.7 Tape: 1-inch wide Time Tape, Professional Tape Co., Riverside, Ill.

1.3.2.3.8 Paper wipers: See 1.1.2.8

1.3.2.3.9 Cardboard cartons: Egg boxes, old-fashioned, upright partitioned, flat bottom, containing 2 eggs X 6 eggs. Vendor: Standard Box Co., P. O. Box 266, Chelsea, Mass.

1.3.2.3.10 Morgue: Any widemouthed vessel having more than 50-ml volume

1.3.2.3.11 Scissors

1.3.3.0 Specifications for cleansing and drying the glassware

1.3.3.1 Detergents or soaps must <u>never</u> be used; they act as insecticides. Any residual detergent or soap left on glassware, or on any surface with which the Habrobracon come in contact, is toxic and lethal

1.3.3.2 The shell vials are placed in the 6 X 6 X 6 inch wire baskets for washing. Each basket contains 49 shell vials

1.3.3.3 Dishes are washed by placing them upside down on trays (1.3.2.2.3)

1.3.3.4 The covers are distributed onto the trays (1.3.2.2.3) made for them

1.3.3.5 The standard cycle is 20 min for the Habrobracon glassware in the Better Built Turbomatic described in paragraph 1.3.2.2.1. During this time the glassware is given 20 rinses (16 of hot tap water and 4 of hot distilled water) under high pressure

1.3.3.6 The bottle-washer brush is not ordinarily used. It is useful for a preliminary cleaning of the shell vials and Stender dishes if the water pressure in the dish washer is too low, or if the water is not hot enough or on long enough to thoroughly clean them. When the bottle-washer brush is employed or a test-tube brush is manually used, the water should be cool or lukewarm, not hot. Again, detergent or soap is <u>not</u> used as a cleaning aid

1.3.3.7 Drying: The glassware is transferred in the baskets to the drying oven for 45 min at 170°F. If the temperature is higher or the time prolonged, the dishes become crazed. This renders them fragile and no longer usable.

1.3.4.0 Etching of shell vials: The shell vials are etched from the top to a distance 1-1/2 cm from the top for half the circumference. The procedure used is as follows:

1.3.4.1 Apply thin film of Miracle Etching Cream with a Q-tip applicator. The film is placed from the top of the vial half-way around the vial and 1/2 inch in height

1.3.4.2 Leave film on vial for 3-5 min

1.3.4.3 Remove film by washing in clear warm water

1.3.5.0 As an alternative procedure to etching the vials, labels (1.3.2.3.2) can be attached just below the lip of the vials

1.3.6.0 After washing and drying, the shell vials are placed in the large trays in storage cabinets or in front of each experimenter

1.3.7.0 Trimmed paper wipers (1.1.2.8) are placed on the bottom of each aluminum pan. Covered Stender dishes are placed in each pan. Time Tape (1.3.2.3.7) is placed on the end of each pan. The pans containing the Stender dishes are then stored for use by experimenters

1.3.8.0 Construction of shell-vial stoppers: No commercially available stopper for the shell vials has been found that is satisfactory for Habrobracon culture. Foam plastic plugs can be used in emergencies, but these have only too often been found to be toxic to the Habrobracon. On the other hand, it has been found that those made manually from cheesecloth and cotton can be used repeatedly until they virtually disintegrate. Stoppers are made by the following procedure:

1.3.8.1 The cheesecloth is cut in pieces 4 inches square

1.3.8.2 The square piece is placed over the top of the vial and then pushed in about 1-1/2 inches

1.3.8.3 Cotton is stuffed into the cheesecloth inside the vial

1.3.8.4 As the cotton is packed in place, the cheesecloth is slowly pulled up evenly to eliminate wrinkles and to tighten the cotton. The plug when finished holds about 1 inch of packed cotton

1.3.8.5 Twelve inches of thread is doubled and wrapped twice around the tightened plug. The thread is tied with a double knot

1.3.8.6 Excess thread and cheesecloth is cut away with scissors

1.3.8.7 The plugs can be used indefinitely if they are sterilized at intervals

1.3.9.0 Assembly of cardboard cartons (1.3.2.3.9) for shell vials: It has been found that shell vials are easily managed in cardboard cartons during experiments. The cartons are inexpensive, lightweight, can

be used many times over, and can be discarded for new cartons at any time. The cartons are acquired flattened. They can be unfolded and are easily kept from collapsing by unfolding and inserting the cardboard spreader that goes with each box. The spreader turns the box into a 12-compartmented carton. Four shell vials fit snugly into each compartment

1.3.10.0 The morgue: The dish for this purpose is filled 1/2-3/4 full with mineral oil (any grade). This provides a convenient place to discard Ephestia and Habrobracon that are no longer needed. The morgue may be cleaned by pouring the oil and debris into a toilet, flushing it with plenty of water, scrubbing the dish with detergent, rinsing, and drying before again adding mineral oil

1.4. Cleansing and Testing of Modules

1.4. Cleansing and testing of modules

1.4.1.0 Scope: Detergents should never have been used on the modules before they are turned over to the experimenter. Detergents act as powerful insecticides. Cleansing of the modules with detergents had been inadvertently specified in component specifications S6545-01-0001-B. This specification had been carried out by G.E. personnel and their subcontractor before the error in document S6545-01-0001-B was found and corrected. Therefore stringent cleansing requirements became necessary, and these are to be carried out by the experimenter

1.4.1.1 Three alternative cleansing procedures have been developed and tested (1.4.2, 1.4.3, and 1.4.4)

1.4.2.0 Cleansing manually

1.4.2.1 The modules are to be disassembled and placed in 250-ml beakers. No more than ten of each of the housings or caps are to be used in any beaker

1.4.2.2 Coarse cheesecloth is placed over each beaker and held in place with elastic bands

1.4.2.3 Distilled water at a temperature of 120-130°F is introduced into each beaker and allowed to remain for 5 min. The beakers are shaken discontinuously

1.4.2.4 The beakers are inverted at the end of 5 min, drained briefly, and fresh distilled water reintroduced

1.4.2.5 This process is to be repeated approximately 50 times

1.4.2.6 At the end of this time 70% alcohol is introduced into each beaker and the beaker shaken discontinuously

1.4.2.7 The beakers are inverted at the end of the 5-min intervals, drained briefly, and 70% alcohol reintroduced

1.4.2.8 The alcohol rinse is repeated five times

1.4.2.9 The housings and cap assemblies are removed from the beakers and air dried

1.4.3.0 Cleansing with ultrasonic vibration

1.4.3.1 Procedure 1.4.2 is followed except that the beakers containing the disassembled modules in distilled water are placed in an ultrasonic vibrator and the water replaced 25 times

1.4.3.2 The alcohol rinse is repeated three times

1.4.4.0 Cleansing with a component scrubber

1.4.4.1 The modules are disassembled and placed in a component scrubber, which was designed and constructed at the Oak Ridge National Laboratory for this purpose. It operates on the principal of a self-filling and periodic-siphoning pipet washer

1.4.4.2 Five gallons of distilled water is slowly siphoned over the disassembled module components

1.4.4.3 One liter of 95% alcohol is used to rinse the components, after which they are laid out on paper toweling to dry

1.4.5.0 Testing the modules for toxic effects on the Habrobracon

1.4.5.1 Habrobracon are anesthetized and placed in modules according to procedure described in paragraphs 2.7.3.3 and 2.7.3.4

1.4.5.2 The cap assemblies are screwed down finger tight.

1.4.5.3 The Habrobracon are left in the modules at room temperature (or approximately 75°F ± 1°) for 100 hr

1.4.5.4 The Habrobracon are removed at the end of the 100-hr interval and examined for toxic symptoms

1.4.5.5 If toxic symptoms are observed, the cleansing procedures will be repeated and the modules retested for toxicity

1.5. Rearing of Habrobracon

1.5. Rearing of Habrobracon

1.5.1.0 Scope: This instruction comprises the techniques used for rearing Habrobracon stocks

1.5.2.0 Equipment

1.5.2.1 Glass shell vials, labeled (1.3.2.1.1, 1.3.4.0, 1.3.5.0)

1.5.2.2 Cotton plugs (1.3.8)

1.5.2.3 Cartons (1.3.2.3.9)

1.5.2.4 Trays of Stender dishes (1.3.2.1.2, 1.3.2.3.6, 1.3.2.3.7, 1.1.2.8, 1.3.7.0)

1.5.2.5.0 Honey feeder

1.5.2.5.1 Three shell vials are attached together by a rubber band. A few grams of honey are placed in one of the vials, a few milliliters of water are placed in another, and the third is left empty as a holder for a dissecting needle

1.5.2.6 Morgue (1.3.2.3.10 and 1.3.10.0)

1.5.2.7.0 Anesthetizing apparatus

1.5.2.7.1.0 Anesthetizing dish (Fig. 1.5-1)

1.5.2.7.1.1 These dishes are constructed from funnels with molded, fritted glass filters. The bottom of the funnel is cut off, sealed shut, and the bottom flattened. A tube is inserted below the fritted glass filter into the middle of the dish from the side. The outlet of the tube is pointed downward to ensure even distribution of the CO_2. A 1/2- to 3/4-inch slot is placed in the side of the funnel to the edge of the fritted glass for ease in removal of the anesthetized Habrobracon. The height of the dish is usually kept at about 1 inch for the 4-inch dishes and 1/2 inch for the 2-inch dishes

1.5.2.7.1.2 Tubing is connected from the valve selection box to the anesthetizing dish

1.5.2.7.2.0 Anesthetizing Stender dish cover

1.5.2.7.2.1 Two holes are drilled in a Stender dish cover. Two metal tubes, each with a fine-mesh screen, are attached to the holes. One of the holes is the CO_2 inlet, the other the outlet; the screen prevents Habrobracon from escaping when the Stender dish covers are replaced with the anesthetizing cover

1.5.2.7.2.2 Tubing is connected from the valve selection box to the anesthetizing cover

Fig. 1.5 - 1. Anesthetizing Dish.

1.5.2.7.3.0 Anesthetizing needle

1.5.2.7.3.1 A hypodermic needle (# 18) is used for anesthetizing Habrobracon in the shell vials by sticking the needle into the vial, either through the stopper or adjacent to it (Fig. 1.5 - 2)

1.5.2.7.3.2 Tubing is connected from the valve selection box to the anesthetizing needle

Fig. 1.5 - 2. A Hypodermic Needle Is Used for Anesthetizing Habrobracon in the Shell Vials by Sticking the Needle into the Vial.

1.5.2.7.4.0 Valve selection system (Fig. 1.5-3)

1.5.2.7.4.1 This is essentially a Lucite box (9 X 10.5 X 8.5 inches) containing a bubble flask half-filled with water. Two glass outlets extend from near the top of the flask and lead by Tygon tubing to a series of valves extending along both sides of the box. By turning these valves, one or all of the anesthetizing units can be activated. The bubble flask serves to humidify the CO_2 and act as a visual indicator that the system is switched on

1.5.2.7.4.2 Tubing is connected from the water trap to the bubble flask in the valve selection box

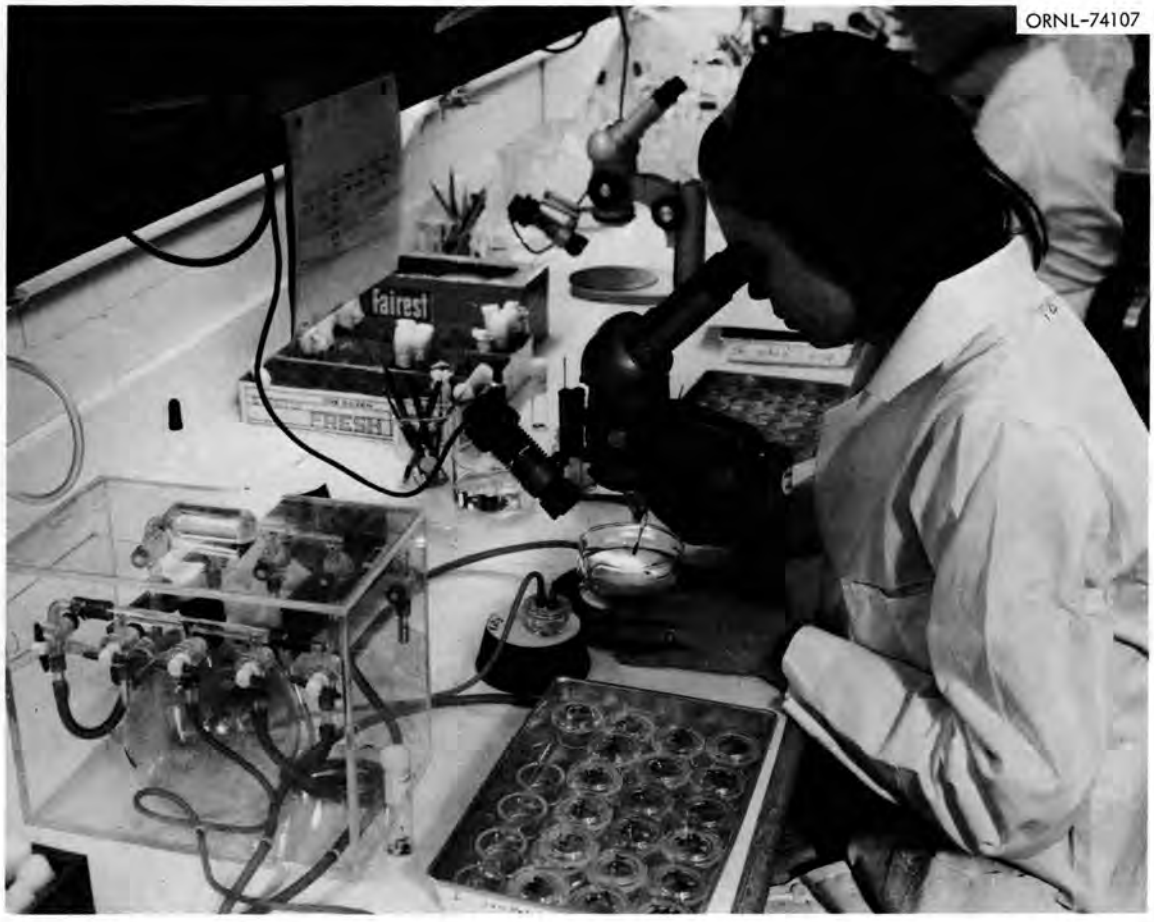


Fig. 1.5-3. The Anethetizing System in Use Under the Microscope.

1.5.2.7.5.0 Water trap

1.5.2.7.5.1 This is an empty flask to keep water from being drawn from the bubble flask into the pressure reduction valve when the apparatus is switched off

1.5.2.7.5.2 Tubing is connected from the pressure reduction valve to the water trap

1.5.2.7.6.0 Pressure reduction valve

1.5.2.7.6.1 Any standard pressure reduction valve can be used to lower the line pressure of the CO_2

1.5.2.7.6.2 The pressure reduction valve is connected either directly to the CO_2 supply or on a metal tubing connection to the CO_2 supply

1.5.2.7.7.0 CO_2 supply

1.5.2.7.7.1 CO_2 is supplied from pressurized tanks

1.5.2.7.7.2.0 For more permanent installations it is convenient to attach tanks in groups to two separate manifolds. The two manifolds are necessary so that the supply can be maintained while empty tanks are being replaced with full ones

1.5.2.7.7.2.1 In the permanent installation, the CO_2 is delivered through pipe lines along the laboratory bench. Each set of anesthetizing apparatus is connected directly to this line

1.5.2.8.0 Stereomicroscope

1.5.2.8.1 We have examined and tried a number of stereomicroscopes in our laboratory at Oak Ridge. Since we routinely make judgments at optical magnifications of 40X, it is essential that the lighting be excellent and the image be the best available. For simplicity of operation and the best all-around performance we have found the Carl Zeiss Stereomicroscope Model II to be unexcelled. This

model is no longer available but Model III is almost as good. The advantage of the Model II is that the two most often used magnifications are 16X and 40X. These are adjacent to one another on the rotary objective system. The advantage of the Model III is that the object is visible at all times while the image size is being changed. The optical quality delivered by the two models is essentially equal

1.5.2.8.2 The lamp systems provided by Carl Zeiss for their microscopes have been found to be convenient, reliable, and bright. It is essential that a heat absorption filter be placed in the light beam, otherwise the biological material rapidly becomes overheated. Two each of the lamp systems are better on each microscope than one since cross-lighting of the object improves identification and makes classification easier

1.5.2.8.3.0 Catalog numbers of the stereomicroscope and accessories

1.5.2.8.3.1 Stereomicroscope Body III with inclined binocular body and Pancratic objective system (47-50-20)

1.5.2.8.3.2 Microscope Stand F (47-52-00)

1.5.2.8.3.3 Stage carrier for Stand F (47-52-30)

1.5.2.8.3.4 Stage with metal insert with ground glass plate (47-52-20)

1.5.2.8.3.5 Eye pieces 10X (46-40-01)

1.5.2.8.3.6 Eye cups (49-49-00)

1.5.2.8.3.7 Lamp holder (46-60-05)

1.5.2.8.3.8 Lamp housing with collector (46-72-55)

1.5.2.8.3.9 Lamp sockets 6v 15w (46-80-12)

1.5.2.8.3.10 Bulbs 6v 15w (38-01-79)

1.5.2.8.3.11 Transformer 3.5-5.5-8v 15VA (39-25-42)

1.5.2.8.3.12 Filter holder (46-60-50)

1.5.2.8.3.13 Heat absorption filter KGI (46-78-30)

1.5.2.9.0 Small instruments

1.5.2.9.1 A number of small instruments are indispensable for investigators handling the Habrobracon. Some of the instruments are easily obtainable anywhere; others less obtainable are described in more detail since they have been found to be extremely useful

1.5.2.9.2.0 Inscribing equipment

1.5.2.9.2.1 No. 2 lead pencils

1.5.2.9.2.2 Sharpie Finepoint Markers (assorted colors). Vendor: Crestwell Supply Co., Knoxville, Tenn.

1.5.2.9.2.3 Pentel fine line, bamboo tipped, black marking pens

1.5.2.9.3.0 Habrobracon handling equipment

1.5.2.9.3.1 Dissecting needles, straight and curved points

1.5.2.9.3.2 Camel's-hair brushes

1.5.2.9.3.3 Large rubber stopper (# 15), preferably white, although covering the stopper with white tape is adequate. This is used for knocking the Habrobracon to the bottom of the dish or vial before the dish is uncovered or the vial is unstoppered

1.5.2.9.3.4 Forceps for handling the Ephestia larvae. These must be very soft so that the animal is uninjured by the handling. Tweezers, thin, nonmagnetic brass, 4-1/2 inches long. Vendor: Southern Tool Distributing Co., 54 11th Street NE, Atlanta, Ga. These have been found to be adequate after the sharply pointed ends have been dulled

1.5.2.9.3.5 Dental probes. These have been found to be extremely useful for dissecting Habrobracon pupae from their cocoons. Explorer probe # 1, Vendor: S. S. White Dental Mfg. Co., Philadelphia, Pa.

1.5.2.10.0 Warm-rooms

1.5.2.10.1 The optimum temperature for maintaining rapidly breeding stocks of Habrobracon is 30°C. For this it was found that such a temperature could be maintained inexpensively in a small room to within 0.1-0.2°C by the use of a sensitive thermoregulator and relay attached to a small room heater. An electric fan helps to distribute the heated air. As soon as the walls of the room also reach a temperature of 30°C, the room temperature is quite stable and the repeated entrances and exits that are necessary during a day's work do not depress the temperature appreciably. We routinely use two heater assemblies and two electric fans in case of failure of one of them. One warm-room is kept at 60% humidity, especially for the hatchability studies; the other warm-room is not humidified but stays at approximately 35% humidity

1.5.2.10.2.0 Warm-room equipment

1.5.2.10.2.1 Thermoregulator: Any mercury thermoregulator will do, but an easily adjustable one is preferred

1.5.2.10.2.2 Electronic relay. Vendor: Precision Scientific Co., Chicago, Ill., Catalog No. 62690

1.5.2.10.2.3 Room heater: Thermador automatic heater

1.5.2.10.2.4 Electric fan: Hassock fans. Vendor: Will Scientific Co., Inc., Catalog No. 51500

1.5.2.10.2.5 Shelving: Adjustable shelving manufactured and sold by W. R. Ames Co., Milpitas, Calif.

1.5.2.10.2.6 Humidifier: Junior Industrial Humidifier, Junior Model SW-5. Vendor: Abbeon Incorporated, 179-15A Jamaica Ave., Jamaica 32, N.Y.

1.5.3.0 Operations

1.5.3.1.0 Maintenance in Stender dishes

1.5.3.1.1 When egg counts and hatchability studies are to be made, each female Habrobracon is placed with one Ephestia larva in a Stender dish. The experimental data can be temporarily recorded on the paper on the bottom of the pan. The experiment itself is recorded on the tape placed at the end of the pan

1.5.3.1.2 The small camel's-hair brush is used for guiding Habrobracon from one Stender dish to another as well as for spreading anesthetized Habrobracon adults in order that they can be observed and counted. The morgue is for discarded Habrobracon and Ephestia

1.5.3.1.3 At 30°C the Habrobracon egg hatches in 29 hr and a generation is obtained in 10 days

1.5.3.2.0 Maintenance in vials

1.5.3.2.1 Adult Habrobracon can be fed by dipping a dissecting needle in honey, then in water, and placing the diluted honey on the inside wall of the vial. They can be stored for several months in the refrigerator. This is an advantage in stock keeping. Before storage the adults should be fed honey. After each month of storage they should be removed from the refrigerator, fed fresh dilute honey and returned. Females feed on host larvae and on honey, males on the latter only

1.5.3.2.2 A female to be set for breeding is placed in a vial with two to five full-grown Ephestia larvae. The vials are placed in the warm-room or kept at room temperature. The Ephestia are paralyzed in a few hours, and eggs are laid within 2 days. The Habrobracon larvae pupate after 3-5 days. After the eggs begin to hatch, the females can be transferred by use of the camel's-hair brush into successive vials labeled with the date, the stock, and the transfer letter — a, b, c, etc. Fresh hosts are added. As many as 300-400 progeny are obtainable from one female if desired

1.5.3.3.0 Collection of virgins

1.5.3.3.1 Virgin females are secured by opening cocoons when they are about to emerge and isolating them. They can be identified within the cocoons, which have been spun on the glass wall of the vial or Stender dish, and easily distinguished from the males by the ovipositor and shorter antennae

1.5.3.4.0 Progeny counts

1.5.3.4.1 When counts are to be made of adults or they are to be scanned for mutations, they are anesthetized either by the use of ether or carbon dioxide. Before anesthetizing, adults should be observed for behavioral mutations, such as "slow motion," "nervous," etc., which would be overlooked after anesthesia

1.5.3.4.2 Since eggs are deposited on the surface of the host, they are easily counted after the adult female has been transferred. Each dish is then returned to the warm-room and the hatchability scored. Time of death of unhatched embryos is checked after 30-36 hr and then recorded. Adult survival is recorded after 10 days

1.6. Testing of Stock for Spontaneous Lethal Mutations

1.6. Testing of stock for spontaneous lethal mutations

1.6.1.0 Scope: This instruction comprises the procedures used for establishing the cultures of Habrobracon free from spontaneous recessive lethal mutations, or with a pedigree so that spontaneous recessive lethal mutations that may be present can be traced familially to determine whether they occurred prior to the experiment

1.6.2.0 Stock cultures

1.6.2.1 Any one Habrobracon stock is normally kept concurrently in four shell vials at room temperature. The stock is shifted twice or thrice weekly to new vials. Fifteen to thirty adults are obtained from each vial, half males and half females

1.6.2.2 When a stock is selected for a large experiment, the cultures are moved to the warm-room and daily changes are made, usually in Stender dishes instead of vials

1.6.2.3 The females are set singly in Stender dishes with a single host and transferred twice daily, or once daily with two hosts

1.6.2.4 From these cultures approximately 24, 48, or 72 virgins are collected

1.6.3.0 Testing of virgins

1.6.3.1 These virgins are kept in vials in groups of ten to twenty and are fed for 2 days at 30°C on several host larvae

1.6.3.2 At the end of the 2-day period the larvae are removed and the virgins are starved overnight. The starvation permits storage of many mature eggs

1.6.3.3 The virgins are set singly with one host each and are transferred twice daily for 2 days (four transfers)

1.6.3.4 The eggs are counted after each transfer and the number is recorded under each dish

1.6.3.5 After the four transfers, the females are transferred to dishes without host larvae and then stored in the refrigerator

1.6.3.6 The hatchabilities are scored 30-36 hr after the eggs are counted. At this time the egg counts and hatchabilities, and times of death are recorded on data sheets

1.6.3.7 After 10 days adult survival is recorded, as are time of death of Habrobracon dying in post-embryonic stages

1.6.3.8 At this time those virgins heterozygous for recessive lethals are eliminated

1.6.3.9 Those females not heterozygous for recessive lethal mutations or chromosomal translocations can be used for small experiments

1.6.3.10 Nevertheless, though they have been kept in the refrigerator, they are about 2 weeks old. Often it is necessary to have very large numbers of newly emerged females for an experiment

1.6.4.0 Pedigrees

1.6.4.1 The virgins which have high hatchability and adult survival are either pair-mated with sons or mass-mated with their sons and sons of all other high-viability females

1.6.4.2 These mated females are set singly in dishes and moved once or twice daily, often for as many as 10 days

1.6.4.3 These females are periodically remated

1.6.4.4 Virgins are collected from each of the lines of females

1.6.4.5 These virgins can be grouped together

1.6.4.6 Testing of a large number of these virgins according to procedure 1.6.3.0 would provide an accurate measurement of the spontaneous mutation frequency

1.6.4.7 If these virgins are needed for an experiment involving matings, accurate records must be maintained on daughters of these females. If any of the females is heterozygous for a spontaneous mutation, half her daughters will be heterozygous also

2. Pre-Flight Operations at Cape Kennedy and Hickam Field

2.1. Equipment and Biological Material Provided by Experimenter

2.1. Equipment and biological material provided by experimenter

2.1.1.0 Scope: This instruction is a checklist for materials needed at the launch and recovery sites. These are the materials supplied by the experimenter and in most cases brought from the Oak Ridge National Laboratory. Some items (e.g., paper wipers, soap, tape) occasionally need to be replenished or purchased on location

2.1.2.0 Cape Kennedy

2.1.2.1.0 General equipment

	Item	Number	Status
2.1.2.1.1	Valve selection box	2	
2.1.2.1.2	Stereomicroscope	4	
2.1.2.1.3	Electrical adaptors	12	
2.1.2.1.4	Beakers — 600 ml	2	
	400 ml	2	
	250 ml	2	
	150 ml	1	
2.1.2.1.5	100-ml Graduate cylinder	1	
2.1.2.1.6	Reducing valves (CO_2)	2	
2.1.2.1.7	1-1/8 inch Combination wrench	1	
2.1.2.1.8	Aluminum pans	81	
2.1.2.1.9	Stender dishes	1944	

2.1.2.2.0 Small supplies

	Item	Number	Status
2.1.2.2.1	Test-tube brushes	2	
2.1.2.2.2	Red Felt-Riters	7	

	Item	Number	Status
2.1.2.2.3	Black Felt-Riters	10	
2.1.2.2.4	Magic transparent tape	6 rolls	
2.1.2.2.5	Time Tape	6	
2.1.2.2.6	Labels	4 boxes	
2.1.2.2.7	Brushes	10	
2.1.2.2.8	18G Hypodermic needles	12	
2.1.2.2.9	Retractable forceps	3 pairs	
2.1.2.2.10	Glassine envelopes	500	
2.1.2.2.11	Used glass-rod dosimeters	40	
2.1.2.2.12	New glass-rod dosimeters	200	
2.1.2.2.13	Glass-plate dosimeters	20	
2.1.2.2.14	Plastic positioners for glass rods	400	
2.1.2.2.15	Scissors	4 pairs	
2.1.2.2.16	Habrobracon score sheets	1 box	
2.1.2.2.17	Tablets	3	
2.1.2.2.18	Razor blades	1 box	
2.1.2.2.19	Plastic squeeze bottle	1	
2.1.2.2.20	Honey feeder	1	
2.1.2.2.21	Anesthetizing dishes	8	
2.1.2.2.22	Anesthetizing lids	3	
2.1.2.2.23	Mineral oil	1 quart	
2.1.2.2.24	Xylene	1 liter	

	Item	Number	Status
2.1.2.2.25	Ephestia larvae collection dishes	2	
2.1.2.2.26	Brass forceps	6 pairs	
2.1.2.2.27	Glass vials	3000	
2.1.2.2.28	Corn meal	50 lb	
2.1.2.2.29	Shirt boxes	24	
2.1.2.2.30	Egg cartons	48	
2.1.2.2.31	Dissecting needles	6	
2.1.2.2.32	Cotton plugs	1000	
2.1.2.2.33	Kimwipes	4 boxes	
2.1.2.2.34	Cheesecloth	1 box	
2.1.2.2.35	Cotton (1 lb)	1 box	
2.1.2.2.36	Scott wipers	3 boxes	
2.1.2.2.37	Masking tape	3 rolls	
2.1.2.2.38	Hand soap	3 bars	
2.1.2.2.39	Rubber tubing	36 feet	
2.1.2.2.40	30°C Room		
2.1.2.2.40.1	Hassock fans	2	
2.1.2.2.40.2	Thermoregulator	1	
2.1.2.2.40.3	1650w Heaters	2	
2.1.2.3.0	Small items for Biosatellite assembly		

	Item	Number	Status
2.1.2.3.1	Torque wrenches	2 sets	
2.1.2.3.2	Spanner wrenches	4	

	Item	Number	Status
2.1.2.3.3	Brass control separator tampers	7	
2.1.2.3.4	Flight loading boards	2 sets	
2.1.2.3.5	CI, CII, CIII loading boards	1 set	
2.1.2.3.6	Component hardware boards	4	
2.1.2.3.7	Component scrubber	1	
2.1.2.3.8	Complete set of FTI's	1	
2.1.2.3.9	Loading layout sheet	1	
2.1.2.3.10.0	Checklists		
2.1.2.3.10.1	Flight	2 sets	
2.1.2.3.10.2	Control I	2 sets	
2.1.2.3.10.3	Control II	2 sets	
2.1.2.3.10.4	Control III	2 sets	
2.1.2.3.11.0	Dosimetry maps		
2.1.2.3.11.1	Flight	2 sets	
2.1.2.3.11.2	Flight back-up	2 sets	
2.1.2.3.11.3	Control I	2 sets	
2.1.2.3.12.0	Habrobracon flight-data sheets		
2.1.2.3.12.1	Flight	2 sets	
2.1.2.3.12.2	Control I	2 sets	
2.1.2.3.12.3	Control II	2 sets	
2.1.2.3.12.4	Control III	2 sets	
2.1.2.3.13	Torque-wrench calibration certificate	1	

2.1.2.4.0 Biological materials

	Item	Number	Status
2.1.2.4.1.0	Ephestia cultures		
2.1.2.4.1.1	Adults	20 bags	
2.1.2.4.1.2	Full-grown larvae	20 boxes	
2.1.2.4.2.0	Habrobracon		
2.1.2.4.2.1	Pretested R-strain stocks	100	
2.1.2.4.2.2	Pretested lemon-strain virgin females	100	
2.1.2.4.2.3	c ho virgins	50	
2.1.2.4.2.4	Lemon males	50	

2.1.3.0 Hickam Field

2.1.3.1.0 General equipment

	Item	Number	Status
2.1.3.1.1	Valve selection box	2	
2.1.3.1.2	Stereomicroscope	2	
2.1.3.1.3	Electrical adaptors	12	
2.1.3.1.4	Reducing valves (CO_2)	2	
2.1.3.1.5	1-1/8 inch Combination wrench	1	
2.1.3.1.6	Aluminum pans	60	
2.1.3.1.7	Stender dishes	1440	
2.1.3.1.8	Portable CO_2 case	1	

2.1.3.2.0 Small supplies

	Item	Number	Status
2.1.3.2.1	Test-tube brush	1	
2.1.3.2.2	Red Felt-Riters	4	
2.1.3.2.3	Black Felt-Riters	4	
2.1.3.2.4	Time Tape	3	
2.1.3.2.5	Labels	2 boxes	
2.1.3.2.6	Brushes	8	
2.1.3.2.7	18G Hypodermic needles	4	
2.1.3.2.8	Retractable forceps	1 pair	
2.1.3.2.9	Glassine envelopes	500	
2.1.3.2.10	Scissors	1 pair	
2.1.3.2.11	Habrobracon score sheets	300	
2.1.3.2.12	Tablets	3	
2.1.3.2.13	Plastic depression trays	100	
2.1.3.2.14	Razor blades	1 box	
2.1.3.2.15	Plastic squeeze bottle	1	
2.1.3.2.16	Honey feeder	1	
2.1.3.2.17	Anesthetizing dishes	4	
2.1.3.2.18	Mineral oil	1 quart	
2.1.3.2.19	Ephestia larvae collection dishes	2	
2.1.3.2.20	Brass forceps	6 pairs	
2.1.3.2.21	Glass vials	400	

	Item	Number	Status
2.1.3.2.22	Corn meal	24 lb	
2.1.3.2.23	Egg cartons	12	
2.1.3.2.24	Dissecting needles	6	
2.1.3.2.25	Cotton plugs	400	
2.1.3.2.26	Kimwipes	2 boxes	
2.1.3.2.27	Scott wipers	2	
2.1.3.2.28	Masking tape	1 roll	
2.1.3.2.29	Hand soap	2 bars	
2.1.3.2.30	Rubber tubing	36 ft	
2.1.3.2.31.0	30°C Room		
2.1.3.2.31.1	Hassock fans	2	
2.1.3.2.31.2	Thermoregulator	1	
2.1.3.2.31.3	1650w heaters	2	
2.1.3.3.0	Small items for Biosatellite disassembly		

	Item	Number	Status
2.1.3.3.1	Torque wrenches	1 set	
2.1.3.3.2	Spanner wrenches	2	
2.1.3.3.3	Brass control separator tamper	1	
2.1.3.3.4	Flight loading boards	1 set	
2.1.3.3.5	Component scrubber	1	
2.1.3.3.6	Complete set of FTI's	1	
2.1.3.3.7	Dosimetry maps — Flight	2	
2.1.3.3.8	Habrobracon flight-data sheets	10	

2.1.3.4.0 Biological materials

	Item	Number	Status
2.1.3.4.1.0	Ephestia cultures		
2.1.3.4.1.1	Adults	6 bags	
2.1.3.4.1.2	Full-grown larvae	12 boxes	
2.1.3.4.2.0	Habrobracon		
2.1.3.4.2.1	R-strain virgin females	400	

2.2. Equipment Provided by NASA

2.2. Equipment provided by NASA

2.2.1.0 Scope: This instruction is a checklist for materials needed at the launch and recovery sites. These are materials provided by NASA

2.2.2.0 Cape Kennedy

2.2.2.1.0 General equipment

	Item	Number	Status
2.2.2.1.1	Laboratory	2	
2.2.2.1.2	Warm-room	1	
2.2.2.1.3	Shelving in warm-room	60 ft	
2.2.2.1.4	Tables	5	
2.2.2.1.5	Desk	1	
2.2.2.1.6	Chairs	9	
2.2.2.1.7	Refrigerators	2	
2.2.2.1.8	Recording thermometer and barometer	1	

2.2.2.2.0 Supplies

	Item	Number	Status
2.2.2.2.1	Charged CO_2 cylinders	4	
2.2.2.2.2	95% Alcohol	on request	
2.2.2.2.3	Distilled water	on request	

2.2.2.3.0 Supplies for Biosatellite assembly

	Item	Number	Status
2.2.2.3.1	FTI and checklist	2	
2.2.2.3.2	Flight hardware	1 set plus 3 spares	

2.2.3.0 Hickam Field

2.2.3.1.0 General equipment

	Item	Number	Status
2.2.3.1.1	Laboratory	1	
2.2.3.1.2	Warm-room	1	
2.2.3.1.3	Shelving in warm-room	40 ft	
2.2.3.1.4	Tables	2	
2.2.3.1.5	Chairs	6	
2.2.3.1.6	Refrigerators	2	
2.2.3.1.7	Recording thermomenter and barometer	1	

2.2.3.2.0 Supplies

	Item	Number	Status
2.2.3.2.1	Charged CO_2 cylinders	2	
2.2.3.2.2	95% Alcohol	on request	
2.2.3.2.3	Distilled water	on request	

2.3. Handling the Habrobracon and Ephestia

2.3. Handling the Habrobracon and Ephestia

2.3.1.0 Scope: This instruction comprises the manipulations needed to obtain sufficient young Habrobracon for the flight

2.3.2.0 Ephestia

2.3.2.1 On T-14, Ephestia are transported to Cape Kennedy either in sandwich bags (when young) or boxes

2.3.2.2 Twenty boxes are either consolidated into about twelve boxes or carried as they are. If consolidated, they are separated upon arrival at Cape Kennedy

2.3.2.3 The Ephestia in bags are handled according to procedure 1.1.

2.3.2.4 The Ephestia boxes are kept refrigerated and procedure 1.2. is followed when Ephestia are needed

2.3.3.0 Habrobracon

2.3.3.1 On T-43, the Raleigh (R) wild-type strain of the Habrobracon and the lemon strain of Habrobracon are taken from stock in Oak Ridge, and mated females transferred twice daily in dishes for 2-5 days

2.3.3.2 On T-19, virgin females (approximately 100) are taken from these dishes and set for hatchability and adult survival measurements according to procedure 1.6.3.0

2.3.3.3 On T-27, females (approximately 100) from the lemon stock are set for six to eight transfers and virgin females collected

2.3.3.4 On T-15, after elimination of females heterozygous for embryo-recessive lethality or translocations, and homozygous for sterility factors, the R females are mated and pedigrees are established according to procedure 1.6.4.0

2.3.3.5 On T-14, these mated R females and the virgin lemon females are transferred to Cape Kennedy

2.3.3.6 On T-14, the Habrobracon females from both cultures are set and transferred twice daily until T-7

2.3.3.7 On T-10, if the culturing is proceeding well, transfers from T-14 and T-13 are brought out to develop at room temperature

2.3.3.8 On T-9, information on females containing larval- and pupal-recessive lethals is telephoned to Cape Kennedy

2.3.3.9 Beginning on T-4, virgin females from the R strain are collected and stored in the refrigerator until preparation for flight begins

2.3.3.10 Beginning on T-4, males from the lemon strain are collected and stored in the refrigerator until preparation for flight begins

2.3.3.11 A major effort is made to obtain all of the males for the flight from cultures that mature at T-30 hr to T-24 hr

2.3.3.12 Virgin R females to be mated post-flight to the lemon males on the flight are transferred refrigerated from Cape Kennedy to Hickam Field by experimenter

2.3.3.13 Numbers of Habrobracon required for the flight and ground-based controls:

Number of males and females to be loaded for launch

	Packages					
	Control		Irradiated		Subtotals	
	♂	♀	♂	♀	♂	♀
Flight	120	120	160	160	280	280
Flight spares	120	120	80	80	200	200
Control I	120	120	160	160	280	280
Control II	120	120	80	80	200	200
Control III	120	120	80	80	200	200
Grand Total					1160	1160

2.4. Cleansing of Culture Glassware and Modules

2.4 Cleansing of culture glassware and modules

2.4.1.0 Scope: This instruction comprises the cleansing of culture glassware and experimental modules at Cape Kennedy and Hickam Field

2.4.2.0 Cleansing of culture glassware

2.4.2.1 The Stender dishes and shell vials are soaked in cool tap water for several hours

2.4.2.2 The inside of each Stender dish or shell vial is scrubbed with a test-tube brush

2.4.2.3 The glassware is thoroughly rinsed in clean tap water

2.4.2.4 A final rinse of the glassware is done in distilled water if sufficient supply is available

2.4.2.5 Stender dishes are stacked on a layer of paper towels to dry. Shell vials are placed in baskets (see 1.3.2.2.2) to dry. A drying oven is used if one is available

2.4.3.0 Cleansing of experimental modules (See Instruction 1.4.4)

2.5. Dosimetry Preparation

2.5. Dosimetry preparation

2.5.1.0 Scope: This instruction comprises the preliminary operations necessary for the dosimetry by means of fluorescence of silvered glass rods

2.5.2.0 Equipment

2.5.2.1 The Toshiba glass rods are purchased from Tokyo Shibaura Electric Co., Ltd., Tokyo, Japan. The jobber is Mitsui Trading Company, 530 Fifth Avenue, New York, N.Y.

2.5.2.2 Toshiba glass blocks, which offer a larger surface with correspondingly better sensitivity are purchased from Tokyo Shibaura Electric Co. (See 2.5.2.1 for address)

2.5.2.3 Plastic string (Teflon spaghetti tubing), AWG size 26 (0.036 O.D.), is purchased from Almac Plastics, Newark, N.J.

2.5.2.4 Glassine envelopes, No. M6-1126, 1-3/4 X 2-3/4 inches, are purchased from Elmore Office Supply Co., Knoxville, Tenn.

2.5.2.5 Self-closing forceps: Trident self-holding with ultrafine points for microdissection; stainless steel; catalog number V-37392

2.5.3.0 Preparation of glass rods and blocks

2.5.3.1 The rods and blocks arrive from the manufacturer in sealed plastic boxes with silica gel enclosed to keep the humidity low

2.5.3.2 The boxes are opened. The glass rods are handled individually with the retractable forceps

2.5.3.3 The glass rods and blocks are individually examined for chipped edges or scratches

2.5.3.4 The chipped and scratched rods and blocks are discarded

2.5.3.5 The inspected rods and blocks are again sealed in the boxes and the silica gel is replaced

2.5.4.0 Preparation of plastic inserts

2.5.4.1 The black plastic string is cut off in lengths of 3.5 mm; 104 are needed

2.5.4.2 The black inserts are used to hold the glass rod centered in the middle dosimeter hole of each module

2.5.4.3 The red plastic string is cut in lengths of 2.0 mm; 208 are needed

2.5.4.4 The red inserts are used to hold the glass rods centered in the two outer dosimeter holes of each module

2.5.4.5 The inserts are kept in two (one for each color) glassine envelopes until needed for the dosimeter loading operation

2.6. Pre-Flight Preparation of Habrobracon

2.6. Pre-flight preparation of Habrobracon

2.6.1.0 Scope: This instruction includes the feeding of Habrobracon females prior to loading into the modules. It also includes the instructions for the preirradiation of one group of males and females

2.6.2.0 Feeding schedule

2.6.2.1 At T-50 hr, three to six Ephestia larvae are placed into the shell vials containing ten to twenty virgin Habrobracon females

2.6.2.2 The Habrobracon females are placed into a 30°C ± 1° incubator or warm-room

2.6.2.3 At T-25 hr the Ephestia larvae are removed from the shell vials

2.6.2.4 The Habrobracon females are gathered in groups of 22 ± 1 per vial and a drop of honey-water mixture is applied to the inner wall of each shell vial

2.6.2.5 The Habrobracon females are placed into a 30°C ± 1° incubator or warm-room until time of loading

2.6.3.0 Irradiation schedule

2.6.3.1 At T-730 ± 30 min each vial of Habrobracon females (22 ± 1) and males to be preirradiated is loaded into a plastic tube (15 mm X 8 mm) and plugged at each end with cotton

2.6.3.2 These Habrobracon are exposed to 2000 R X-rays

2.6.3.3 After the exposure each group of females and males is placed into a labeled vial preparatory to placing them in the modules

2.7. Preparation and Assembly of the Biosatellite Package

2.7. Preparation and assembly of the Biosatellite package (Fig. 2.7-1)

2.7.1.0 Scope: This instruction covers the insertion of the Habrobracon experiment into flight and ground control hardware and the subsequent assembly of this hardware

Fig. 2.7-1. Preparation and Assembly of the Biosatellite Package

2.7.2.0 Equipment

2.7.2.1 CO_2 supply: Bottled compressed gas; pressure reducing valve; back pressure flasks; anesthetizing set (1.5.2.0)

2.7.2.2 Habrobracon and Ephestia manipulation (see 1.5.2.0): Needles, camel's-hair brushes, forceps, stereomicroscopes, lamps, rubber stoppers, vials and plugs, module positioning boards

2.7.2.3 Dosimetry equipment: Glass rods, glass plates, reverse action forceps, glassine envelopes, and Pentel marking pens (2.1.2.2.0)

2.7.2.4 Torque wrenches and screwdrivers (2.1.2.3.0)

2.7.2.5 Habrobracon Biosatellite hardware: Upon receiving all hardware from bonded storage, a complete inventory is taken to make sure that all the components can be accounted for and that they meet specifications

2.7.3.0 Preparation for insertion of the Habrobracon experiment into the Biosatellite hardware

2.7.3.1 The module housings are placed into the module postioning boards. The identification number found on each housing and its position on the module positioning board is recorded on the loading checklist (2.7.6, Appendix B)

2.7.3.2 The module caps, screens, inserts, and control caps are placed on the component hardware trays. Each tray contains the exact number of hardware components necessary for loading each module positioning board

2.7.3.3 After the module positioning boards and component hardware trays are loaded, they are arranged at the appropriate stations for assembly of the Habrobracon packages

2.7.3.4 The brackets of the Habrobracon packages are ordered according to the loading schedule (see checklist) at the appropriate stations

2.7.4.0 Insertion of the Habrobracon experiment into the Biosatellite hardware. See Appendix A (2.7.5) for step-by-step operations

2.7.4.1 Biological dosimeters are inserted into each bracket assembly (T-1500 to T-1080)

2.7.4.2 The biological dosimeters are secured in place by using the cap plugs for this purpose. Each plug is torqued down to 20 ± 2 inch oz

2.7.4.3 One glass rod dosimeter is inserted into each of the three holes provided in each housing. Each dosimeter is held in position with plastic inserts

2.7.4.4 Vials of Habrobracon adults (2.5 and 2.6) are arranged in order of loading at each station. Each vial is labeled according to the module or module layer into which the Habrobracon will be placed

2.7.4.5 All loading equipment and Habrobracon adults are laid out at each station according to Fig. 2.7-2 by T-710 min

2.7.4.6 Adult Habrobracon are placed onto a CO_2 dish (1.5.2.0) after first being anesthetized within the shell vial. One shell vial of Habrobracon is anesthetized and loaded into the module before the next vial is handled

2.7.4.7 The number of anesthetized Habrobracon to be brushed into each module or module layer is 15 (+5, -3) (Fig. 2.7-3 and 2.7-4)

2.7.4.8 Cap assemblies are started over the threads of the module housings (finger tight). The loading of control modules is different (See Appendix A)

2.7.4.9 The cap assemblies are torqued down to 24 ± 2 inch oz by the adjacent investigator who is responsible for this procedure (See Fig. 2.7-5 for general flow plan for loading)

2.7.4.10 The modules shall be installed finger tight into the bracket assembly (Fig. 2.7-6)

2.7.4.11 The modules are then torqued to 32 ± 2 inch oz (T-640)

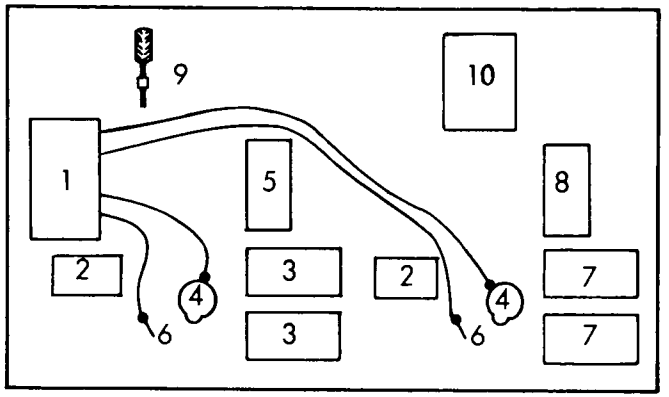
2.7.4.12 The packages are inspected for any errors in assembly and the dosimeter positions are mapped (2.7.7, Appendix C)

2.7.4.13 The electron equilibrium shields are attached to the designated bracket assemblies. The screws used for the attachment are torqued to 25 ± 2 inch oz (T-625)

2.7.4.14 All packages are transferred to General Electric personnel for installation into the Biosatellite vehicle

Phase I

Loading Habrobracon



Phase II

Package Assembly

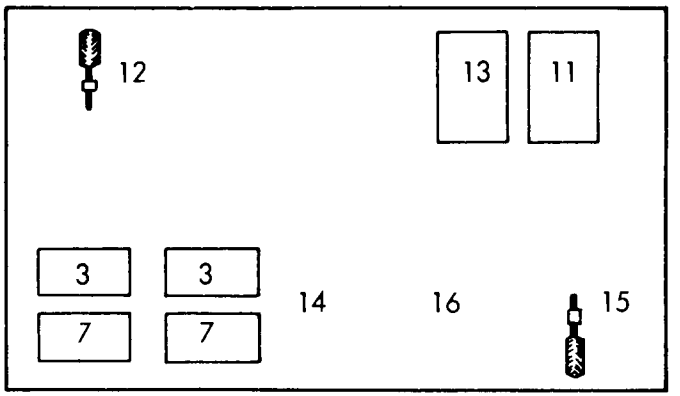


Fig. 2.7-2. Table Layout for Loading Habrobracon

1. CO_2 manifold
2. Component hardware tray
3. Positioning board – females
4. CO_2 dish
5. Habrobracon females
6. Anesthetizing needle
7. Positioning board – males
8. Habrobracon males
9. Torque wrench – 24 ± 2 inch oz
10. Checklists – loading Habrobracon into modules
11. Dosimetry maps
12. Torque wrench – 32 ± 2 inch oz
13. Checklists – placing modules into brackets; attachment of radiation shields
14. Brackets
15. Torque wrench – 24 ± 2 inch oz
16. Radiation shields

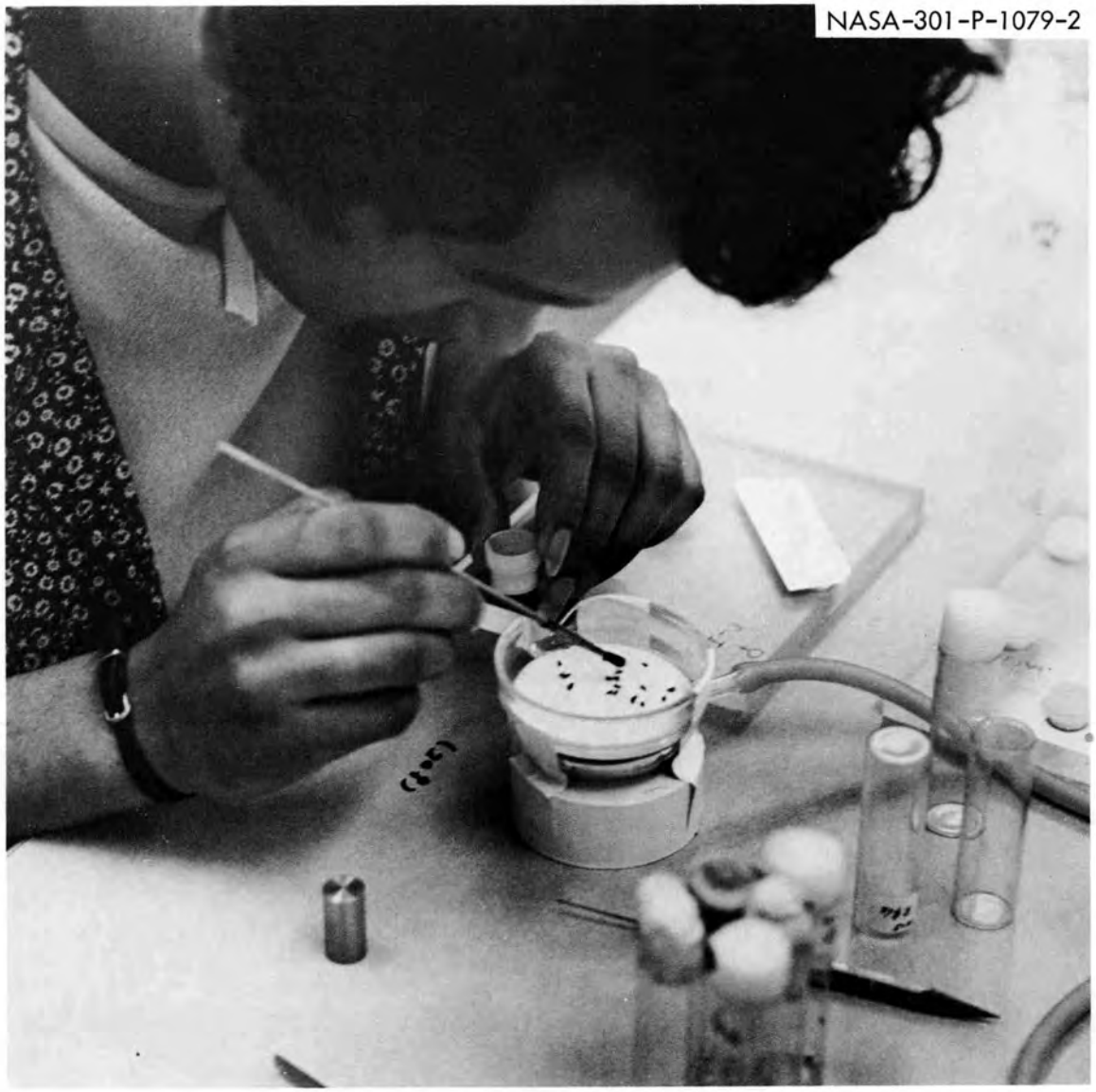


Fig. 2.7-3. Adult Habrobracon Are Brushed from a CO_2 Dish into Each Module

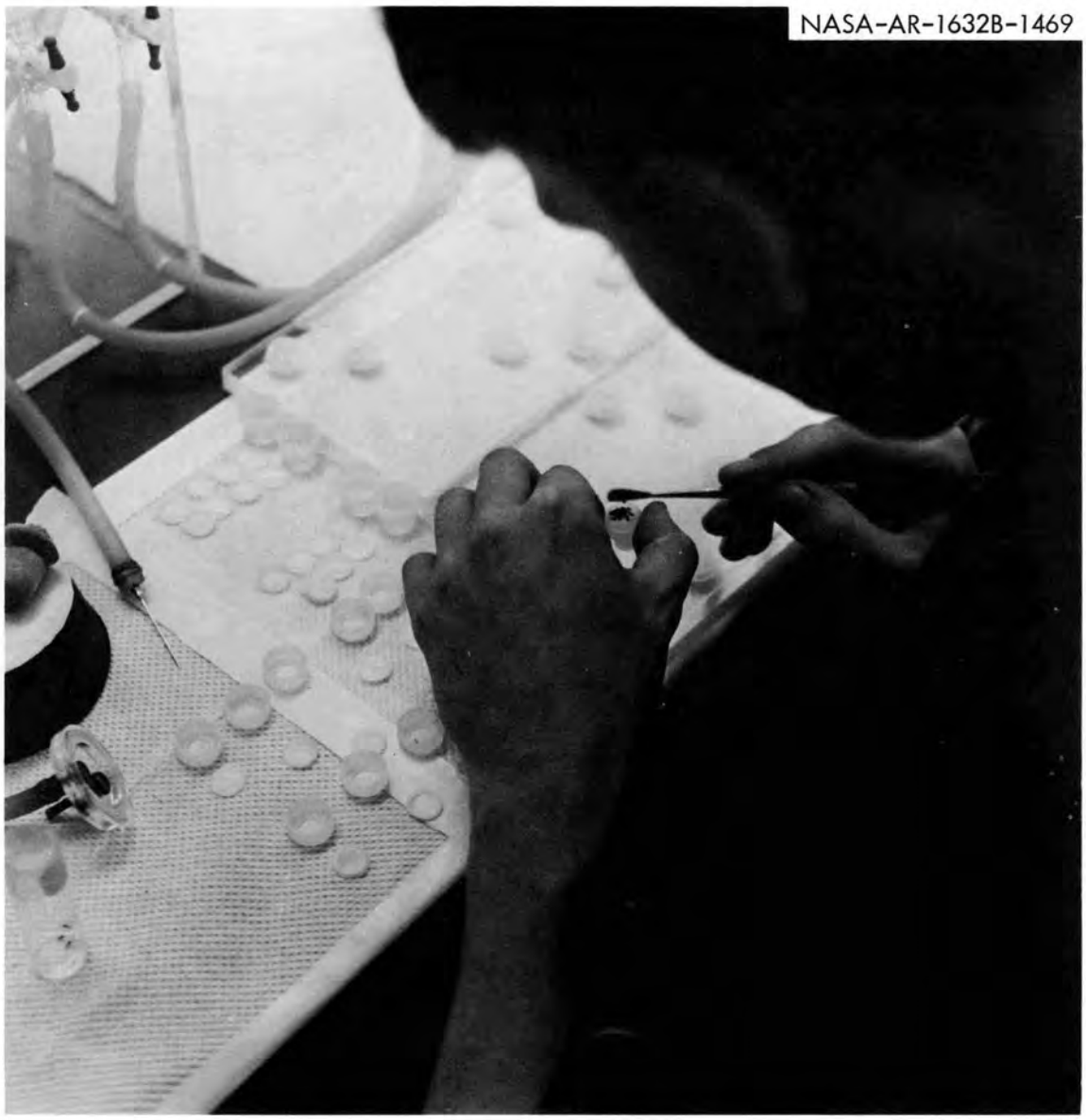
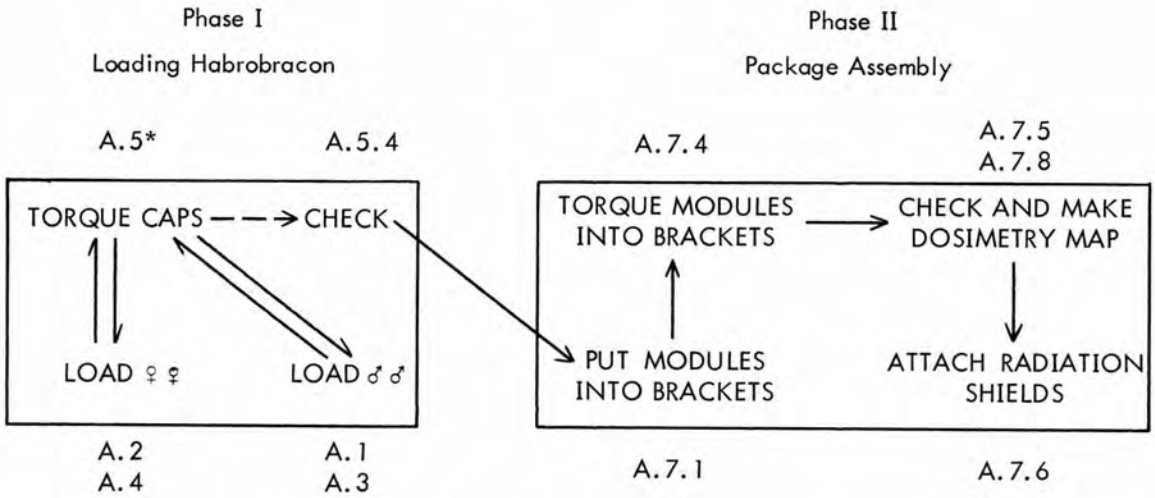


Fig. 2.7-4. Habrobracon Females Being Brushed into the Module.

The layout of screens, separators, modules, and caps can be seen. The module-positioning board is in the background.



*Operations are given in Instruction 2.7.5 (Appendix A)

Fig. 2.7-5. Flow Plan for the Assembly of the Habrobracon Biosatellite Packages.
(The numbers refer to flight-time instructions)

Fig. 2.7-6. The Modules Are Installed Finger Tight Into the Bracket Assembly.

2.7.5. Appendix A — Habrobracon Loading Operations

2.7.5. Appendix A — Habrobracon Loading Operations

This instruction is a step-by-step description of the loading of adult Habrobracon into the Biosatellite hardware. All previous operations have been completed up to 2.7.4.6. Operations A.1 and A.2 are carried out simultaneously

A.1.0 The loading of adult male Habrobracon (lemon strain) into modules

A.1.1 The male Habrobracon [15 (+5, -3)] contained in the first shell vial are anesthetized and emptied into the anesthetizing dish. All shell vials are labeled according to the modules into which the Habrobracon are to be placed

A.1.2 The male Habrobracon are brushed into the upper left (UL) module of the first package (No. 1). The module must be removed from the module-positioning board for this operation

A.1.3 A screen is placed over the Habrobracon and a cap is threaded finger tight over the module housing

A.1.4 The loaded module is returned to its original position in the module-positioning board; a check mark is placed on the board beside the module, indicating that this module has been loaded and is ready to be to be torqued down

A.1.5 Steps A.1, 2, 3, 4 are repeated for the lower right (LR) module housing of package No. 1.

A.1.6 Steps A.1, 2, 3, 4, 5 are repeated for flight packages Nos. 2, 3, 4, Sp No. 1, Sp No. 2; ground control I packages Nos. 1, 2, 3, 4; ground control II and ground control III packages (3 packages each)

A.2.0 The loading of adult female Habrobracon (Raleigh strain) into modules

A.2.1 The female Habrobracon [15 (+5, -3)] contained in the first shell vial are anesthetized and then emptied into the anesthetizing dish. All shell vials are labeled according to the modules into which the Habrobracon are to be placed

A.2.2. The Habrobracon are brushed into the upper right (UR) module of the first package (No. 1). The module must be removed from the module positioning board for this operation (Fig. 2.7-4)

A.2.3 A screen is placed over the Habrobracon and a cap is threaded finger tight over the housing

A.2.4 The loaded module is returned to its original position in the module-positioning board; a check mark is placed on the board beside the module, indicating that this module has been loaded and is ready to be torqued down

A.2.5 Steps A.2.1, 2, 3, 4, are repeated for the lower left (LL) module housing of package No. 1

A.2.6 Steps A.2.1, 2, 3, 4, 5 are repeated for flight packages Nos. 2, 3, 4, Sp No. 1, Sp No. 2; ground control I packages Nos. 1, 2, 3, 4; ground control II and ground control III packages (3 packages each)

A.3.0 The loading of adult male Habrobracon into the control package modules (Fig. 2.7-3)

A.3.1 Insert the first separator into a control cap

A.3.2 Brush 15 (+5, -3) anesthetized Habrobracon into the control cap

A.3.3 Insert a second separator into the same control cap

A.3.4 Brush 15 (+5, -3) anesthetized Habrobracon into this second layer of the control cap

A.3.5 Insert a third separator into the same control cap

A.3.6 Brush 15 (+5, -3) anesthetized Habrobracon into this third layer

A.3.7 Place a screen over this third layer of Habrobracon

A.3.8 Remove the UL control module housing for flight control package No. 5 from the module-positioning board and thread it into the control cap. Do not lose glass dosimeters from the housing

A.3.9 Return the loaded control module to its original position in the board; make a check mark on the board beside the loaded module to indicate that this module is ready to be torqued down

A.3.10 Repeat steps A.3.1 through A.3.9 for the loading of LR control module of control flight package, the Sp C package, the ground control I control package

A.4.0 The loading of adult female Habrobracon into the control package modules (Fig. 2.7-4)

A.4.1 Steps A.3.1 thru A.3.10 are repeated for the UR and LL control modules of the flight control package, the Sp C package, and the ground control I control package

A.5.0 The torquing down of caps to the module housings

A.5.1 The module-positioning boards are passed to the person in charge of torquing when the two modules from the same package containing Habrobracon of the same sex have been loaded and checked off as ready for torquing (Fig. 2.7-2). This same person torques down modules containing male and female Habrobracon

A.5.2 The modules have each cap assembly torqued down to 24 ± 2 inch oz

A.5.3 The person in charge of torquing circles the check mark next to the torqued module to indicate that this module is ready to be installed into the bracket assembly

A.5.4 The inspector records the time (real time) that the two modules are torqued

A.5.5 The module-positioning board containing the torqued modules is passed back to the station whose Habrobracon are being loaded

A.5.6 Steps A.5.1 through A.5.5 are repeated until all modules are loaded and torqued down

A.5.7 When all module-positioning boards are completed, the inspector arranges the boards for the next operation

A.6.0 The four experimenters take their positions for the following operations (Fig. 2.7-2)

A.7.0 The installation of the modules into the bracket assemblies

A.7.1 An experimenter takes the UL module from the No. 1 position in the module-positioning board and threads it finger tight into the upper left position of the No. 1 bracket assembly (when facing the front). As he does this operation, he calls out the module number to the inspector who checks this number with the one recorded on the Flight Data Sheet (2.7.8, Appendix D) when the glass-rod dosimeters were loaded

A.7.2 Operation A.7.1 is repeated for the UR, LR, and LL modules (clockwise)

A.7.3 When the operation is completed for the first package, it is passed to the person in charge of torquing

A.7.4 Each module is torqued down to 32 ± 2 inch oz in the same clockwise direction, UL → LL

A.7.5 When the torquing is completed for this package, the inspector records the time on the checklist

A.7.6 The bracket assembly containing the modules is passed on for attachment of the radiation shields

A.7.7 Operations A.7.1 through A.7.6 are repeated until all modules are placed into the bracket assemblies

A.7.8 The inspector checks each package and records the positions of the glass-rod dosimeters on the dosimeter map (2.7.7, Appendix C)

A.8.0 The attachment of the radiation shields to the bracket assemblies

A.8.1 The radiation shields are installed finger tight with the two screws provided

A.8.2 Each screw is torqued down to 24 ± 2 inch oz

A.8.3 The inspector records the time at which each radiation shield installation is completed

A.8.4 Operations A.8.1 through A.8.3 are repeated until all packages requiring radiation shields are completed (packages No. 1, 2, 3, 4 from the flight and ground control groups)

(Operations A.7 through A.8 are done in assembly line fashion)

2.7.6. Appendix B — Checklists

CHECKLIST: FLIGHT

Loading Habrobracon into Modules

Torque: 24 ± 2% inch lb

Date:

Programmed time: Start T-700 Finish T-640

Actual time: Start Finish

MODULES: UL, LR	TIME	INITIALS
1		
2		
3		
4		
C		
Sp 1		
Sp 2		
Sp C		

CHECKLIST: FLIGHT

Loading Habrobracon into Modules

Torque: 24 ± 2% inch lb

♀

Date:

Programmed time: Start T-700 Finish T-640

Actual time: Start Finish

MODULES: UR, LL	TIME	INITIALS
1		
2		
3		
4		
C		
Sp 1		
Sp 2		
Sp C		

CHECKLIST: FLIGHT

Placing Modules into Brackets

Torque: 32 ± 2% inch lb

Date:

Programmed time: Start T-640 Finish T-625

Actual time: Start Finish

PACKAGE	TIME	INITIALS
1		
2		
3		
4		
C		
Sp 1		
Sp 2		
Sp C		

CHECKLIST: FLIGHT

Attachment of Radiation Shields

Torque: 24 ± 2% inch lb

Date:

Programmed time: Start T-625 Finish T-610

Actual time: Start Finish

PACKAGE	TIME	INITIALS
1		
2		
3		
4		
C	*****	*****
Sp 1		
Sp 2		
Sp C	*****	*****

CHECKLIST: CONTROL I

Loading Habrobracon into Modules ♂

Torque: 24 ± 2% inch lb

Date:

Programmed time: Start T-700 Finish T-640

Actual time: Start Finish

MODULES: UL, LR	TIME	INITIALS
1		
2		
3		
4		
C		

CHECKLIST: CONTROL I

Loading Habrobracon into Modules

Torque: 24 ± 2% inch lb

Date:

Programmed time: Start T-700 Finish T-640

Actual time: Start Finish

MODULES: UR, LL	TIME	INITIALS
1		
2		
3		
4		
C		

CHECKLIST: CONTROL I

Placing Modules into Brackets

Torque: 32 ± 2% inch lb

Date:

Programmed time: Start T-640 Finish T-625

Actual time: Start Finish

PACKAGE	TIME	INITIALS
1		
2		
3		
4		
C		

CHECKLIST: CONTROL I

Attachment of Radiation Shields

Torque: 24 ± 2% inch lb

Date:

Programmed time: Start T-625 Finish T-610

Actual time: Start Finish

PACKAGE	TIME	INITIALS
1		
2		
3		
4		
C	******	******

CHECKLIST: CONTROL II

Loading Habrobracon into Modules ♂

Torque: 24 ± 2% inch lb

Date:

Programmed time: Start T-700 Finish T-640

Actual time: Start Finish

MODULES: UL, LR	TIME	INITIALS
1		
2		
3		

CHECKLIST: CONTROL II

Loading Habrobracon into Modules

Torque: 24 ± 2% inch lb

♀

Date:

Programmed time: Start T-700 Finish T-640

Actual time: Start Finish

MODULES: UR, LL	TIME	INITIALS
1		
2		
3		

CHECKLIST: CONTROL II

Placing Modules into Brackets

Torque: 32 ± 2% inch lb

Date:

Programmed time: Start T-640 Finish T-625

Actual time: Start Finish

PACKAGE	TIME	INITIALS
1		
2		
3		

CHECKLIST: CONTROL III

Loading Habrobracon into Modules

Torque: 24 ± 2% inch lb

Date:

Programmed time: Start T-700 Finish T-640

Actual time: Start Finish

MODULES: UL, LR	TIME	INITIALS
1		
2		
3		

CHECKLIST: CONTROL III

Loading Habrobracon into Modules

Torque: 24 ± 2% inch lb

Date:

Programmed time: Start T-700 Finish T-640

Actual time: Start Finish

MODULES: UR, LL	TIME	INITIALS
1		
2		
3		

CHECKLIST: CONTROL III

Placing Modules into Brackets

Torque: 32 ± 2% inch lb

Date:

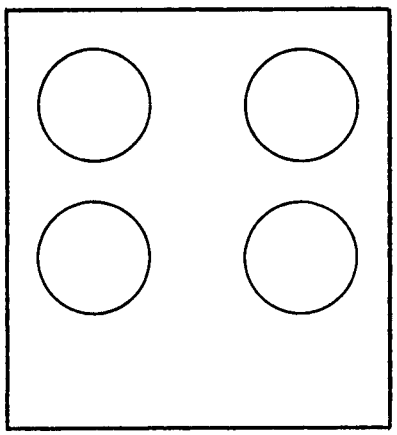
Programmed time: Start T-640 Finish T-625

Actual time: Start Finish

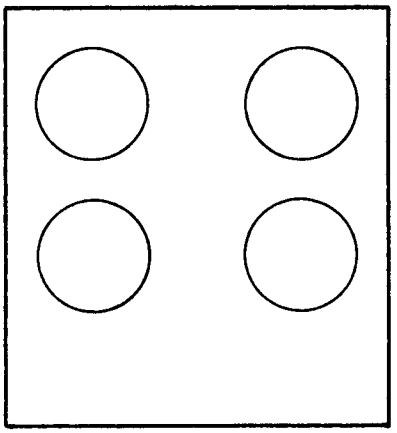
PACKAGE	TIME	INITIALS
1		
2		
3		

2.7.7. Appendix C — Dosimetry Maps

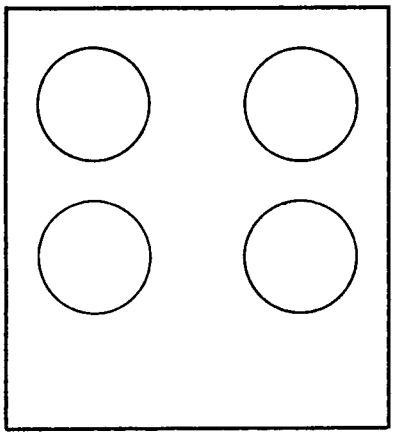
DOSIMETRY MAP
Prime Flight



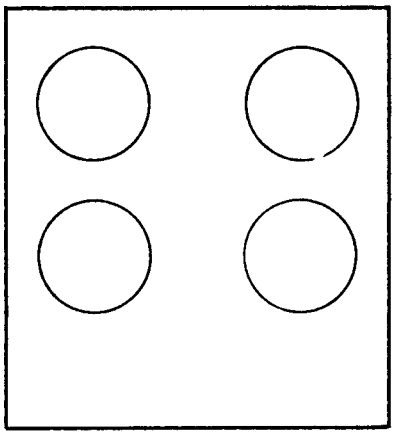
F 1



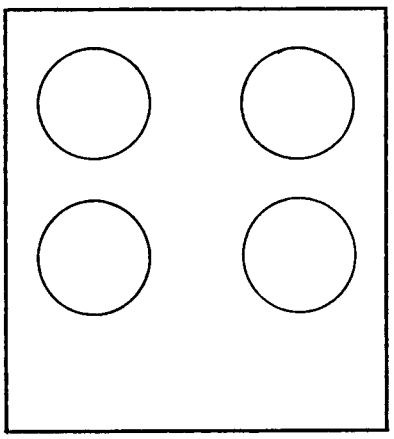
F 2



F 3



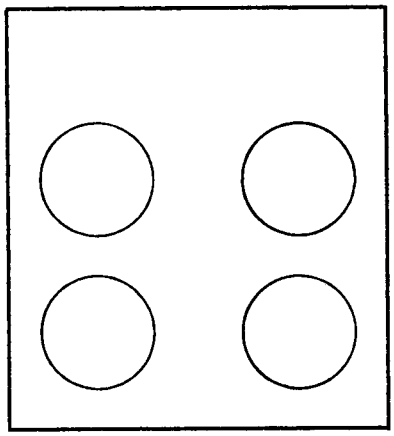
F 4



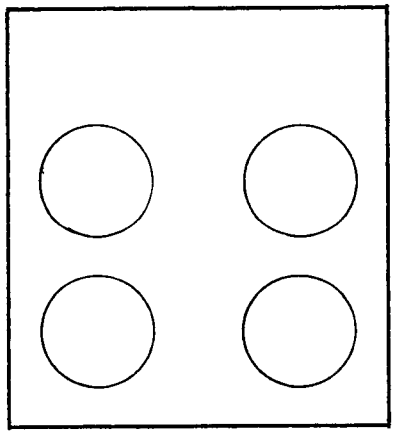
A 1

DOSIMETRY MAP

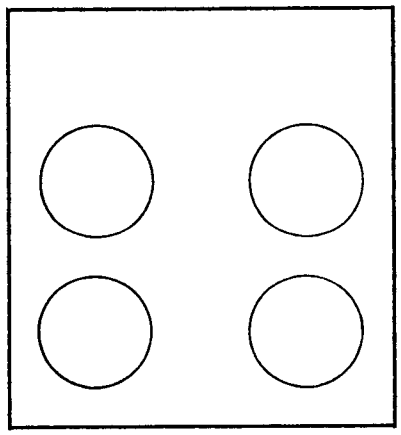
Flight Backup



G 1



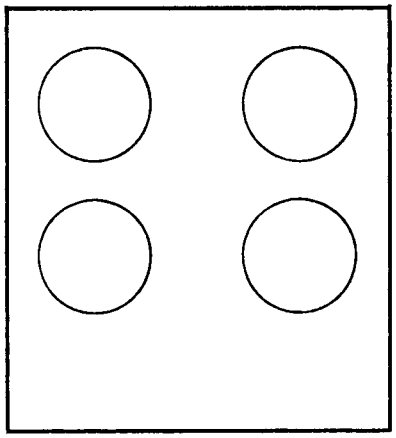
G 3



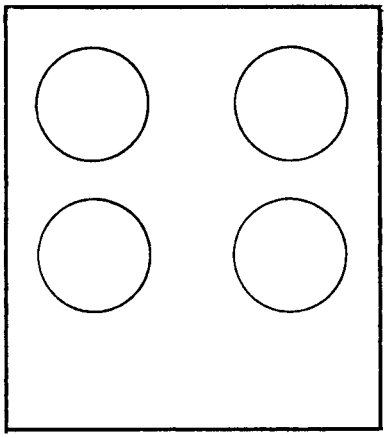
G 4

DOSIMETRY MAP

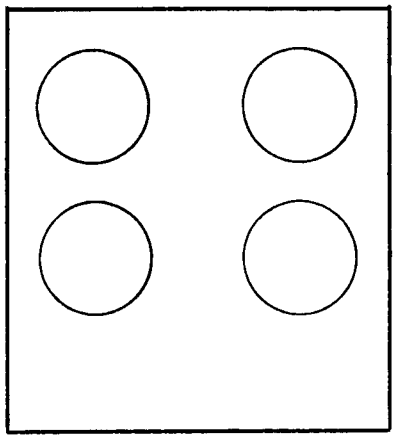
Control I



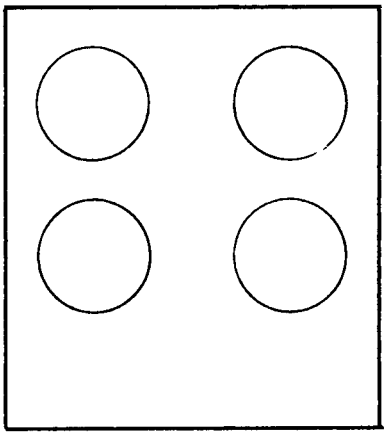
1



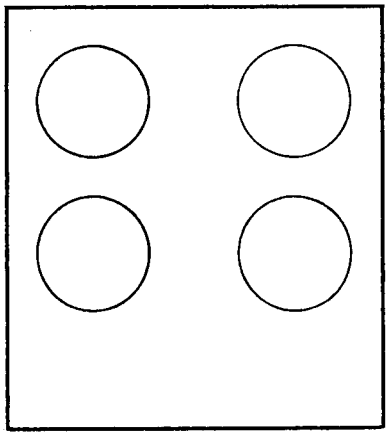
2



3



4



5

2.7.8. Appendix D — Habrobracon Flight Data Form

HABROBRACON FLIGHT DATA

DATE

PACKAGE NO. | NOMINAL DOSE

MOD. POS.	MOD. NO.	DOSIMETERS PRE-READING	DOSIMETERS POST-READING	SEX	NO. WASPS	NO. SURVIVORS	COMMENTS
UL		OUTSIDE (REF)					
		CENTER					
		CENTER					
		OUTSIDE					
UR		OUTSIDE (REF)					
		CENTER					
		CENTER					
		OUTSIDE					
LR		OUTSIDE (REF)					
		CENTER					
		CENTER					
		OUTSIDE					
LL		OUTSIDE (REF)					
		CENTER					
		CENTER					
		OUTSIDE					

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2.7.9. Appendix E — Flight-Time Instructions from General Electric

TASK C - EXPERIMENTER FTI (HABROBRACON)

Time			Verified
	1.0	Phased Preparations of Habrobracon and Assembly of the Biosatellite Package.	
	1.1	This instruction covers the insertion of the Habrobracon experiment into flight hardware and the subsequent assembly of this hardware component, Drawing 47D169029.	_____
	1.2	Equipment	
	1.2.1	CO_2 supply: Bottled, compressed gas pressure-reducing valves, back-pressure flasks, bubble flasks, tubing, anesthetizing dishes, syringe needles.	_____
	1.2.2	Habrobracon and Ephestia manipulation: Needles camels hair brushes, forceps, stereomicroscopes, lamps, rubber stoppers, module-positioning boards, vials and plugs, Esterbrook Felt-riter marking pens.	_____
	1.2.3	Dosimetry equipment: Glass rods, glass plates, reverse action forceps, glassine envelopes and Pentel marking pens.	_____
	1.2.4	Torque wrenches and screwdrivers.	_____
T-1500 to T-1800	1.3	Preparation of Dosimeters	
	1.3.1	Two biological dosimeters will be inserted in assembly.	_____
	1.3.2	The biological dosimeters will be secured in place using the cap plugs provided for this purpose. The cap plugs shall be torqued down to 20 $\pm$ 2 in. oz. Measured torque _____.	_____
	1.3.3	Three (3) glass dosimeters will be inserted into the 3 holes provided in the threaded portion of each housing. Each dosimeter will be held stationary with plastic inserts.	_____
	1.4	Preflight Irradiation of Habrobracon	
	1.4.1	Habrobracon are anesthetized and placed in plastic tubes with cotton plugs closing both ends.	_____

'TI 239952 B

'ASK C (continued)

'ime			Verified
:-730±30	1.4.2	Plastic tubes containing the Habrobracon are irradiated with an exposure of 2000 roentgens.	_____
	1.5	Preparation of Biological Material and Package Assembly	
	1.5.1	Vials checked for dead or sick Habrobracon before anesthetization.	_____
:-700	1.5.2	Adult Habrobracon shall be placed onto an opened dish which has humidified CO_2 atmosphere for anesthetization.	_____
	1.5.3	The number of anesthetized wasps to be brushed into every module housing or separator is to be 15 + 5, -3.	_____
	1.5.4	Cap assemblies will be started over the threads of the module housings.	_____
	1.5.5	The cap assemblies shall be torqued down to 24 ± 2 in. oz.	_____
[-640	1.5.6	The modules shall be installed finger tight into the bracket assembly. The modules are then torqued to 32 ± 2 in. oz.	_____
	1.5.7	The electron equilibrium shield shall be attached to the package (bracket assembly). The screws used for the attachment shall be torqued to 24 ± 2 in. oz.	_____

PACKAGE NO.	MODULE SERIAL NUMBER
A1 (A810) Serial #_____	UL_____
	UR_____
	LL_____
	LR_____
F1 (A822) Serial #_____	UL_____
	UR_____
	LL_____
	LR_____

FTI 239952 C

TASK C continued

TIME	1.5.7 continued		Verified
	PACKAGE NO.	MODULE SERIAL NUMBER	
	F2 (A820) Serial #_____	UL _____	
		UR _____	
		LL _____	
		LR _____	
	F3 (A821) Serial #_____	UL _____	
		UR _____	
		LL _____	
		LR _____	
	F4 (A823) Serial #_____	UL _____	
		UR _____	
		LL _____	
		LR _____	
	BACK-UP PACKAGE	MODULE SERIAL NUMBER	
	G1	UL _____	
		UR _____	
		LL _____	
		LR _____	
	G3	UL _____	
		UR _____	
		LL _____	
		LR _____	
	G4	UL _____	
		UR _____	
		LL _____	
		LR _____	
T-610	1.6	Transfer eight (8) packages (five (5) flight and three (3) back-up) to G. E.	_____

4.14 TASK 12 - ASSEMBLY OF HABROBRACON PACKAGES

Countdown Time: Start T-440 min. (_____hrs.)
Time for completion of Task: 40 min.

Responsibility			Verified by Inspector
Assembler	1.	Obtain from experimenter five (5) habrobracon assemblies, Pkg. #1, 2, 3, 4, 5, for Control I and three (3) each for Control II (#35, 36, 40) and III (41, 42, 43).	_____
Inspector		1.1 Record time of day at receipt.	
Assembler	2.	Insert two (2) filled LiF dosimeter tubes into their respective slots on Package #1 per figure 11. Torque to 15 ± 2 in. oz.*	_____
Assembler	3.	Insert two (2) filled LiF dosimeter tubes into their respective slots on Package #2 per figure 11. Torque to 15 ± 2 in. oz.*	_____
Assembler	4.	Insert two (2) filled LiF dosimeter tubes into their respective slots on Package #3 per Figure 11. Torque to 15 ± 2 in. oz.*	_____
Assembler	5.	Insert two (2) filled LiF dosimeter tubes into their respective slots on Package #4 per Figure 11. Torque to 15 ± 2 in. oz.	_____
Assembler	6.	Insert two (2) filled LiF dosimeter tubes into their respective slots on Package #5 per Figure 11. Torque to 15 ± 2 in. oz.	_____
Assembler	7.	Attach one (1) glass plate dosimeter in its plastic envelope to either side surface of Package #5 using two layers of 3M Brand Magic Mending Tape. Care should be taken so that the glass plate surface is not scratched.	_____
Assembler	8.	Attach two (2) film packages to Package #5 using two layers of 3M Brand Magic Mending Tape at the following locations.	_____
		8.1 Flat against the top of the package mounting surface. Film Pack #_____.	_____

TASK 12 (continued)

Responsibility			Verified by Inspector
	8.2	Side face opposite side holding glass plate. Film Pack No. _____.	_____
Inspector	9.	Using an ohmeter, measure and record the thermistor resistance values._____.	_____

#1 _____K ohms

2 _____

3 _____

4 _____

5 _____

*NOTE: CARE MUST BE TAKEN SO THAT LiF TUBES ARE NOT BOWED MORE THAN ½ MM AFTER INSTALLATION. IF SO, REPLACE.

4.15 TASK 13 - INSTALLATION AND TEST PROCEDURE FOR HABROBRACON PACKAGES

Countdown Time: Start T-398 Min. (_____hrs).
Time for completion of Task: 32 Min.

Responsibility			Verified by Inspector
Installer	1.	Receive from courier Habrobracon Packages #1 2, 3, 4, and 5.	_____
Inspector		1.1 Record time of receipt.	_____
Installer	2.	Mount packages #1, 2, 3, 4, and 5 to their respective positions. Refer to Figures No. 4 - 5. Torque all screws to 10-12 in. lbs.	_____
		Remove Trad Package to install Habrobracon package #3. Mount Forward Tradescantia package with 6 screws. Torque to 10-12 in. lbs.	_____
		Rotate package #4 fully clockwise before tightening screws.	_____
Installer	3.	Connect wires #5 to Package #5. Torque all screws to 5.5-6 in. lbs.	_____
Installer	4.	Connect wires #1 to Package #1. Torque all screws to 5.5-6 in. lbs.	_____
Installer	5.	Connect wires #2 to Package #2. Torque the screws to 5.5-6 in. lbs.	_____
Installer	6.	Connect wires #3 to Package #3. Torque all screws to 5.5-6 in. lbs.	_____
Installer	7.	Connect wires #4 to Package #4. Torque all screws to 5.5-6 in. lbs.	_____
Inspector	8.	Record the identification numbers of the packages installed below	
		8.1 Package A822 _____	
		8.2 Package A821 _____	
		8.3 Package A820 _____	
		8.4 Package A823 _____	
		8.5 Package A810 _____	
Inspector	9.	Record the time of completion.	_____

2.7.10. Appendix F — Hardware Checklist

HARDWARE CHECKLIST

Package	Bracket Serial Number	Module Assemblies		Screens	Separators	External Dosimeter Screws	Secondary Electron Equilibrium Shield	Screws for Shield	Biological Dosimeter Plugs	Missing Components
		Caps	Housings							
Flight										
G 1										
G 1										
G 1										
G 1 (spare)										
G 3										
G 3 (spare)										
G 4										
G 4 (spare)										
Control I										
1										
2										
3										
4										
5 (control)										
Control II										
1										
2										
3										
Control III										
1										
2										
3										

2.7.11. Appendix G — Preinventory and Preloading Checklist

Preinventory and Preloading Checklist

Item	Status	Missing Components
Flight loading boards		
Control loading boards		
Component hardware trays		
Selected glass rods		
Position inserts for glass rods		
Reverse-action forceps		
Camel's-hair brushes		
Torque wrenches		
Anesthetizing equipment		
Flight-data sheets		
Flight and ground-based control hardware		
General Electric flight-time instructions		

2.8. Calibration of Equipment at Cape Kennedy

2.8. Calibration of equipment at Cape Kennedy

2.8.1.0 Scope: This instruction pertains to the calibration of the refrigerators and the warm-room

2.8.2.0 Equipment

2.8.2.1 1 - Thermohumidograph

2.8.2.2 Thermometers

2.8.3.0 Calibration of warm-room

2.8.3.1 The controls (on thermoregulator and heaters) are adjusted to maintain 86°F ± 2°

2.8.4.0 Calibration of refrigerators

2.8.4.1 Controls are adjusted to maintain 40°F ± 2°. Temperatures checked daily to ensure temperatures are within specifications

2.9. Calibration of Equipment at Hickam Field

2.9. Calibration of equipment at Hickam Field

2.9.1.0 Scope: This instruction pertains to the calibration of refrigerators and incubators at Hickam Field

2.9.2.0 Equipment

2.9.2.1 Thermometers (2)

2.9.3.0 Calibration of incubators

2.9.3.1 Controls are adjusted to maintain 86°F ± 2°. Temperature is recorded every 2 hr for at least 8 hr to ensure constancy

2.9.3.2 Temperature is checked at least twice daily thereafter

2.9.4.0 Calibration of refrigerators

2.9.4.1 Controls are adjusted to maintain 40°F ± 2°. Temperature is checked at least once daily

3. In Flight Operations at Cape Kennedy and Hickam Field

3.1. Labeling Vials and Stender Dish Trays

3.1. Labeling vials and Stender dish trays

3.1.1.0 Scope: This instruction pertains to labeling vials for the collection of Habrobracon from the flight and ground control modules, and the preparation of Stender dishes to begin the post-flight operations

3.1.2.0 The preparation of vials

3.1.2.1 Gummed labels are applied to shell vials (~800). These shell vials are placed into cardboard cartons (~16)

3.1.2.2 Six cartons of vials are placed into an attaché case which is flown to Hickam Field within 24 hr after the launch of the Biosatellite

3.1.2.3 The rest of the shell vials remain at Cape Kennedy to be used for the unloading of the ground control modules

3.1.2.4 These shell vials are labeled during the in-flight waiting period. They are each labeled according to module position and nominal exposure. The vials for the ground-based controls are also labeled according to which control the modules are from

3.1.3.0 The preparation of Stender dishes and trays

3.1.3.1.0 Labels on the trays of Stender dishes are inscribed according to the following sequence:

3.1.3.1.1 At Hickam Field (flight) —
date/ dose level/ package position/ transfer number/ ♀ numbers

3.1.3.1.2 At Cape Kennedy and Oak Ridge (flight ground-based control) —
I, II, or III/ date/ dose level/ package postion/ transfer number/ ♀ numbers

3.1.3.2 The list of labels to be used is in section 3.1.4 (Appendix A)

3.1.3.3 These trays are stacked for use after the Habrobracon females are unloaded and Habrobracon males have been mated to females

3.1.4. Appendix A — Label Listing for Trays of Stender Dishes

Hickam Field:

Females from the flight packages

4000 R	UR		1-24
	LL		25-48
2000 R	UR		49-72
	LL		73-96
1000 R	UR		97-120
	LL		121-144
500 R	UR		145-168
	LL		169-192
pre 2000 R	UR	inner	193-216
		mid	217-240
		outer	241-264
pre 2000 R	LL	inner	265-288
		mid	289-312
		outer	313-336

Cape Kennedy:

Females from the ground-based control packages

Control I

4000 R	UR		337-360
	LL		361-384
2000 R	UR		385-408
	LL		409-432
1000 R	UR		433-456
	LL		457-480
500 R	UR		481-504
	LL		505-528
pre 2000 R	UR	inner	529-552
		mid	553-576
		outer	577-600
pre 2000 R	LL	inner	601-624
		mid	625-648
		outer	649-672

Cape Kennedy:

Females from the ground-based control packages

Control II

pre 2000 R	UR	673-696
0 R	LL	697-720

Control III

pre 2000 R	UR	721-744
0 R	LL	745-768

Packages

41, 42	Forward
43	Aft

3.2. Readout of Environmental Conditions and Plans for Emergencies

3.2. Readout of environmental conditions and plans for emergencies

3.2.1.0 Scope: The reports on all measured conditions of the flight of the Biosatellite are recorded at Goddard Space Flight Center. These reports are forwarded after every orbit to Cape Kennedy, and the information pertinent to the Habrobracon experiment is recorded by a representative of the Habrobracon experiment. A representative from the experiment is on duty 24 hr a day in order to advise in case of emergency and to prepare a continuing collation of the data returned from the Biosatellite and the controls

3.2.2.0 Designation of shifts

3.2.2.1 Three shifts of 8 hr 15 min duration are assigned to the three investigators remaining at Cape Kennedy

3.2.2.2 Experimenters must overlap for 15 min in order to convey new developments and instructions

3.2.2.3 On the third day of flight, the experimenter on the midnight shift is relieved an hour early in order that he may be able to get 6 hr sleep before splashdown

3.2.3.0 At hourly intervals, the experimenter goes from the laboratory to the communications headquarters to receive and record reports

3.2.4.0 When not recording data at the communications headquarters, the experimenter is carrying out activities necessary for post-flight operations

3.2.5.0 Percent data recovery at different times in orbit is presented by the experimenter upon request. For the Habrobracon experiment, this is as follows:

After →	1 hr	10 hr	20 hr	30 hr	40 hr	50 hr	60 hr
Data Returns →	20%	40%	60%	80%	90%	100%	100%

3.2.6.0 Emergency procedures

3.2.6.1 Should an emergency arise on the flight and an early call-down is deemed advisable for technical reasons, the experimenter on duty should vote to bring it down, since after one orbit 20% of the data from the flight are already obtained. The flight pattern must be mimicked with timed procedures on controls. Telephone calls to other experimenters are placed to advise that post-flight operations will begin

3.2.6.2 Should an emergency arise in the ground-based controls, II and/or III, the experimenter on duty should bring the packages from controls II and/or III into the laboratory at room temperature, and move the temperature and humidity recorder onto the same bench as these controls

3.2.6.3 Should an emergency arise in control I and it is deemed necessary to shut down this part of the experiment for technical reasons, the experimenter on duty should vote to keep control I as close as possible to the flight conditions, avoiding shutdown if possible

3.2.6.4 Should there be a need to evacuate the building, the experimenter on duty should collect data books, the Habrobracon females, and one box of Ephestia needed for post-flight operations, and leave the building forthwith

3.3. Preparation of Score Sheets

3.3. Preparation of score sheets

3.3.1.0 Scope: This instruction pertains to the preparation of score sheets for recording data at Cape Kennedy, Hickam Field, and Oak Ridge

3.3.2.0 Habrobracon Score Sheets (see page 7, ORNL-TM-1732) are labeled, numbered, and placed into manila folders. The score sheets and folders are coded to the pans and Stender dishes (3.1) which will contain the experimental Habrobracon males and females

3.3.3.0 This operation is completed at the three locations (Cape Kennedy, Hickam Field, and Oak Ridge) prior to the retrieval of the Biosatellite vehicle

3.4. Preparation of Females for Mating

3.4. Preparation of females for mating

3.4.1.0 Scope: This instruction pertains to the handling of Habrobracon virgin females (strain R) prior to mating with Habrobracon males from the flight and ground control (I, II, and III) experiments

3.4.1.1 Habrobracon females (2.4) are taken to Hickam Field to mate to Habrobracon males from the Biosatellite vehicle. Each vial of females contains host Ephestia larvae. The cardboard boxes of vials are packed into an attaché case for the trip from Cape Kennedy to Hickam Field, and hand carried

3.4.1.2 These Habrobracon females are placed into a refrigerator (6-10°C) as soon as possible upon arriving in Honolulu, Hawaii

3.4.1.3 They will be removed from the refrigerator when the males from the flight are ready to be mated

3.4.1.4 The Habrobracon females to be mated to the control males at Cape Kennedy are kept refrigerated until the males are taken from controls I, II, and III

3.5. Preparation of Ephestia

3.5 Preparation of Ephestia

3.5.1.0 Scope: This instruction comprises the procedure used to obtain paralyzed Ephestia larvae for the post-flight operations involving the experimental females

3.5.2.0 On T-5, 500 Habrobracon females are irradiated in the facilities available at the Oak Ridge National Laboratory

3.5.3.0 These females are given an exposure of approximately 10,000 R

3.5.4.0 The females are well fed with Ephestia larvae and allowed to lay eggs for 3 days at 30°C

3.5.5.0 The females are removed from the vials with the Ephestia larvae into new vials and refrigerated. The Ephestia larvae are discarded

3.5.6.0 On T-3 days, 300 of the females are transported to Cape Kennedy from Oak Ridge

3.5.7.0 On T+1 day, 200 of the females are transported to Hickam Field from Oak Ridge

3.5.8.0 On T+40 hr, the Habrobracon females are divided into groups of about 10-20, and each group of females is given 5-10 Ephestia larvae

3.5.9.0 After 3-5 hr these Ephestia larvae are removed, examined for the presence of eggs (which are removed if found), and the paralyzed Ephestia are refrigerated

3.5.10.0 Instructions 3.5.8 and 3.5.9 are repeated until T+66 or until sufficient Ephestia for post-flight operations are ready

3.5.11.0 For post-flight operations these amount to 940 paralyzed Ephestia that are needed for the experimental Habrobracon from the flight, 940 Ephestia for Control I, 600 for Control II, and 600 for Control III

3.5.12.0 At T+66, these paralyzed Ephestia are placed one each in the Stender dishes to be used in the transfers of females

4. Post-Flight Operations at Cape Kennedy and Hickam Field

4.1. Package Disassembly

4.1. Package disassembly

4.1.1.0 Scope: This instruction comprises the disassembly of the packages and modules and the assigning of radiation monitors and Habrobracon to the appropriate containers

4.1.2.0 Operations

4.1.2.1 Receive packages from General Electric personnel

4.1.2.2 Unscrew electron equilibrium shield and lithium fluoride radiation-measuring assemblies

4.1.2.3 Deliver lithium fluoride radiation-measuring assemblies to Ames Research Center personnel

4.1.2.4 Unscrew modules from bracket assemblies

4.1.2.5 Place modules in carrying racks

4.1.2.6 Deliver loaded carrying racks to investigators

4.1.2.7 Habrobracon are anesthetized by inverting modules on CO_2 anesthetizing dishes

4.1.2.8 Caps of individual modules are unscrewed, each module separately, to release Habrobracon, being careful not to allow glass rods to fall out

4.1.2.9 Habrobracon from one module are brushed into prelabeled vials, and operations 4.1.2.8 and 4.1.2.9 are repeated for next module

4.1.2.10 Operations 4.1.2.8 and 4.1.2.9 are repeated until all modules are emptied

4.1.2.11 Modules are recapped finger tight and placed in carrying racks

4.1.2.12 After the Habrobracon have recovered from anesthetization, they are placed in labeled shell vials and numbers of survivors are recorded

4.1.2.13 Carrying racks are delivered to investigator C for removal of radiation monitors

4.1.2.14 Caps of individual modules are unscrewed, each module separately

4.1.2.15 The monitors are removed into separate prelabeled envelopes

4.1.2.16 The biological and block dosimeters are removed from the bracket assemblies into prelabeled vials and glassine envelopes

4.1.2.17 The modules are cleansed according to procedure 1.4.4 before being returned to bonded storage

4.2. Handling the Habrobracon

4.2. Handling the Habrobracon

4.2.1.0 Scope: This instruction comprises the procedures used for mating the Habrobracon males and setting the Habrobracon females immediately after they have been removed from the modules

4.2.2.0 Habrobracon females

4.2.2.1 The females are anesthetized and brushed individually into dishes with one stung Ephestia each. These dishes are in the trays provided for them

4.2.2.2 After a 4-hr interval the females are transferred to the next set of trays in the series

4.2.2.3 After a 2-hr interval the females are transferred again

4.2.2.4.0 The complete set of transfers with paralyzed Ephestia is as follows:

4.2.2.4.1 A — 4 hr

4.2.2.4.2 B — 2 hr

4.2.2.4.3 C — 2 hr

4.2.2.4.4 D — 12 hr

4.2.2.4.5 E — 12 hr

4.2.2.5 The eggs laid on these Ephestia are counted, hatchabilities taken, and the hatched larvae left for adult survival studies

4.2.2.6 After this interval the females are placed with unparalyzed Ephestia for a 24-hr period

4.2.2.7 The eggs laid on these Ephestia are washed into mineral oil containers for hatchability studies

4.2.2.8 Instructions 4.2.2.6 and 4.2.2.7 are repeated for 20 days except for experiments on day 3 and day 12

4.2.2.9 On days 3 and 12 hatchability studies are made according to instruction 4.2.2.5

4.2.3.0 Habrobracon males

4.2.3.1 A few Habrobracon males are brushed into appropriately labeled vials with individual virgin females

4.2.3.2 The males and females are watched by an observer until they are seen to mate

4.2.3.3 If the female rejects the male, then the female is removed, anesthetized, and placed again with the male

4.2.3.4 A brush or needle is used to guide the male near the female until he senses her presence and copulates

4.2.3.5 After an observed mating, the vial containing the male and female is set aside

4.2.3.6 Procedure 4.2.3.1 is repeated until all Habrobracon are mated

4.2.3.7 The Habrobracon males together with the mated females are transported to Oak Ridge for testing of the mated females

5. Personnel Assignments

5.1. Deployment of Personnel

5.1. Deployment of personnel

5.1.1.0 Scope: This instruction describes the most economical and efficient placement of the Habrobracon investigators in their working capacities at Cape Kennedy, Goddard Space Flight Center, Hickam Field, Raleigh, and Oak Ridge. The assignments of specific individuals are listed in instruction 5.1.6 (Appendix)

5.1.2.0 Cape Kennedy

5.1.2.1 The work at Cape Kennedy prior to flight requires the efforts of two investigators to properly prepare the Habrobracon which are to be used in the ground-based controls and the flight

5.1.2.2 As the flight approaches, more individuals are required for the pre-flight operations

5.1.2.3 On T-3, six more individuals are brought to Cape Kennedy for the final pre-flight operations and the assembly

5.1.2.4 On T+1 days, two of the individuals at Cape Kennedy go to Hickam Field to participate in the post-flight operations there and three go to Oak Ridge for preparation of the laboratory

5.1.2.5 The three people left at Cape Kennedy share the in-flight duties

5.1.2.6 The post-flight operations at Cape Kennedy occupy the three people for 2 days and two people for 4 days, until the experiment is moved to Raleigh and Oak Ridge

5.1.2.7 One person must go to Cape Kennedy on T+15 to make final adult survival analyses on the portion of the experiment left there

5.1.3.0 Hickam Field

5.1.3.1 One individual goes to Hickam Field at T-7 to carry out pre-flight operations and to prepare the laboratory

5.1.3.2 Two persons go from Cape Kennedy on T+1 to participate in in-flight and post-flight operations

5.1.3.3 One half of the experiment remains at Hickam Field for 20 days with three individuals in attendance, and the other half of the experiment is returned to Oak Ridge

5.1.3.4 It may be necessary for one or more persons to transport Ephestia larvae to Hickam Field from T+7 to T+20

5.1.4.0 Goddard Space Flight Center

5.1.4.1 One investigator from the Habrobracon group carries out the operations at Goddard Space Flight Center and is on instant call for decisions regarding the disposition of the Biosatellite should an emergency arise

5.1.4.2 This person arrives at Goddard Space Flight Center on T-2, carries out in-flight operations, and departs on T+6

5.1.5.0 Oak Ridge

5.1.5.1 One person remains at Oak Ridge and carries out the necessary operations for post-flight activities

5.1.5.2 It is important that this person be thoroughly versed in all operations involving Habrobracon and that a state of complete readiness be achieved for glassware, laboratory apparatus, and host Ephestia larvae

5.1.6. Appendix — Position Assignments of Specific Investigators

Habrobracon P-1079

Location of personnel for pre-flight, in-flight, and post-flight operations

O – Oak Ridge
R – Raleigh
C – Cape Kennedy
H – Hickam Field
G – Goddard

	T-12	11	10	9	8	7	6	5	4	3	2	1	Launch 0	T+ 1	2	3	Recovery 4	5	6	7	8	9	10	11	12	13
Amy, R. L.	G	G	G	G	Home							G	G	G	G	G	G	Home								
Baird, M. B.	C	C	C	C	C	C	C	C	C	C	C	C	C	C	O	O	O	O	O	O	O	O	O	O	O	O
Cain, K. T.	C	O	O	O	O	O	O	O	O	C	C	C	C	H	H	H	H	H	H	H	O	O	O	O	O	O
Grosch, D. S.	R	R	R	R	R	R	R	R	R	R	C	C	C	C	C	C	C	R	R	R	R	R	R	R	R	R
Haye, M. J. L.	C	O	O	O	O	O	O	O	O	C	C	C	C	C	O	O	O	O	O	O	O	O	O	O	O	O
Jones, M. S.	C	O	O	O	O	O	O	O	O	C	C	C	C	C	O	O	O	O	H	H	H	H	H	H	H	H
Reel, J. W.	C	O	O	O	O	O	O	O	O	O	C	C	C	C	C	O	O	O	O	O	O	O	O	O	O	O
Smith, R. S.	C	C	C	C	C	C	C	C	C	C	C	C	C	H	H	H	H	O	O	O	O	O	O	O	O	O
Valcovic, L. R.	C	O	O	O	O	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
von Borstel, R. C.	C	O	O	O	O	O	O	O	O	O	C	C	C	C	C	C	C	O	O	O	O	O	O	O	O	O
Whiting, A. R.	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

5.2. Assembly Teams

5.2. Assembly teams

5.2.1.0 Scope: This instruction lists the assignments and names of the investigators during the loading and assembly of the Habrobracon packages for the Biosatellite experiment and its ground-based controls

5.2.2.0 Phase I — Loading of Habrobracon

5.2.2.1.0 Team A: Ground Controls I and II

5.2.2.1.1 R. C. von Borstel (check)

5.2.2.1.2 M. J. Lane (torque caps)

5.2.2.1.3 M. S. Jones (load ♂♂)

5.2.2.1.4 M. B. Baird (load ♀♀)

5.2.2.2.0 Team B: Flight and Ground Control III

5.2.2.2.1 R. H. Smith (check)

5.2.2.2.2 J. W. Reel (torque caps)

5.2.2.2.3 D. S. Grosch (load ♂♂)

5.2.2.2.4 K. T. Cain (load ♀♀)

5.2.3.0 Phase II — Assembly of Package

5.2.3.1.0 Team A: Ground Controls I and II

5.2.3.1.1 R. C. von Borstel (check and make dosimetry map)

5.2.3.1.2 M. S. Jones (attach radiation shield)

5.2.3.1.3 M. J. Lane (torque modules into brackets)

5.2.3.1.4 M. B. Baird (put modules into brackets)

5.2.3.2.0 Team B

5.2.3.2.1 R. H. Smith (check and make dosimetry map)

5.2.3.2.2 D. S. Grosch (attach radiation shields)

5.2.3.2.3 J. W. Reel (torque modules into brackets)

5.2.3.2.4 K. T. Cain (put modules into brackets)

5.3. Deployment for Shift Duties During the Flight

5.3. Deployment for shift duties during the flight

5.3.1.0 Scope: This instruction lists the names of personnel who will be on shift duty at Cape Kennedy during in-flight operations

5.3.2.0 0800-1615 J. W. Reel

5.3.3.0 1600-0015 D. S. Grosch

5.3.4.0 0000-0815 R. C. von Borstel

5.4. Disassembly Teams

5.4. Disassembly teams

5.4.1.0 Scope: This instruction lists the names of personnel involved in disassembly operations and gives their assigned duties

5.4.2.0 Hickam Field

5.4.2.1.0 Disassembly of Habrobracon packages

5.4.2.1.1 R. H. Smith

5.4.2.1.2 L. R. Valcovic

5.4.2.2.0 Setting Habrobracon females from flight

5.4.2.2.1 K. T. Cain

5.4.2.2.2 R. H. Smith (as soon as he has completed disassembly operations)

5.4.2.3.0 Mating of males from flight with virgin females

5.4.2.3.1 L. R. Valcovic (as soon as he has completed disassembly operations)

5.4.2.3.2 K. T. Cain (as soon as she has completed the setting of Habrobracon females from the flight)

5.4.2.3.3 R. H. Smith (as soon as he has completed disassembly operations and the setting of the Habrobracon females from the flight)

5.4.2.4.0 Unloading of radiation exposure detectors

5.4.2.4.1 R. H. Smith

5.4.2.4.2 K. T. Cain

5.4.3.0 Cape Kennedy

5.4.3.1.0 Dissassembly of Habrobracon packages

5.4.3.1.1 R. C. von Borstel

5.4.3.1.2 D. S. Grosch

5.4.3.2.0 Setting Habrobracon females from flight

5.4.3.2.1 J. W. Reel

5.4.3.2.2 R. C. von Borstel (as soon as he has completed disassembly operations)

5.4.3.3.0 Mating of males from flight with virgin females

5.4.3.3.1 D. S. Grosch (as soon as he has completed disassembly operations)

5.4.3.3.2 J. W. Reel (as soon as she has completed the setting of Habrobracon females from the flight)

5.4.3.3.3 R. C. von Borstel (as soon as he has completed disassembly operations and the setting of the Habrobracon females from the flight)

5.4.3.4.0 Unloading of radiation exposure detectors

5.4.3.4.1 R. C. von Borstel

5.4.3.4.2 D. S. Grosch

6. Post-Flight Operations on the Habrobracon Experiment

6.1. The Habrobracon Experiment at Hickam Field, Cape Kennedy, and Raleigh

6.1 The Habrobracon experiment at Hickam Field, Cape Kennedy, and Raleigh

6.1.1.0 Scope: This instruction is a brief description of the operations to be carried out on the experiment on Habrobracon females that are returned in the flight vehicle and on their ground-based control

6.1.2.0 The females are to be transferred in trays according to the following schedule:

6.1.2.1 4 hr

6.1.2.2 2 hr

6.1.2.3 2 hr

6.1.2.4 2 hr

6.1.2.5 12 hr

6.1.2.6 8 hr

6.1.3.0 The females are then to be transferred at 24-hr intervals for 18 days

6.1.4.0 Ten days after the beginning of the experiment, adult survival data are recorded for the hatchability experiment covering the first 2 days

6.1.5.0 After the transfers described in instruction 6.1.2 are carried out at Cape Kennedy, the females are moved in vials to Raleigh. The post-flight operations are continued within 6 hr of the time they are transferred from Cape Kennedy

6.2. The Habrobracon Experiment at Oak Ridge

6.2 The Habrobracon experiment at Oak Ridge (Fig. 6.2-1)

6.2.1.0 Scope: This instruction is a brief description of the operations to be carried out in the experiment on Habrobracon males that are returned from the flight vehicle and on their ground-based control

6.2.2.0 The complete operations are shown in the flow diagram (6.2.6, Appendix)

6.2.3.0 The analyses of the F_2 females are to be carried out in the following order:

	Condition (exposure)	Flight	Ground-Based Control
6.2.3.1	0	500	0
6.2.3.2	2000	400	100
6.2.3.3	0	0	500
6.2.3.4	4000	200	100
6.2.3.5	500	400	100
6.2.3.6	1000	400	100
6.2.3.7	pre-2000	400	100

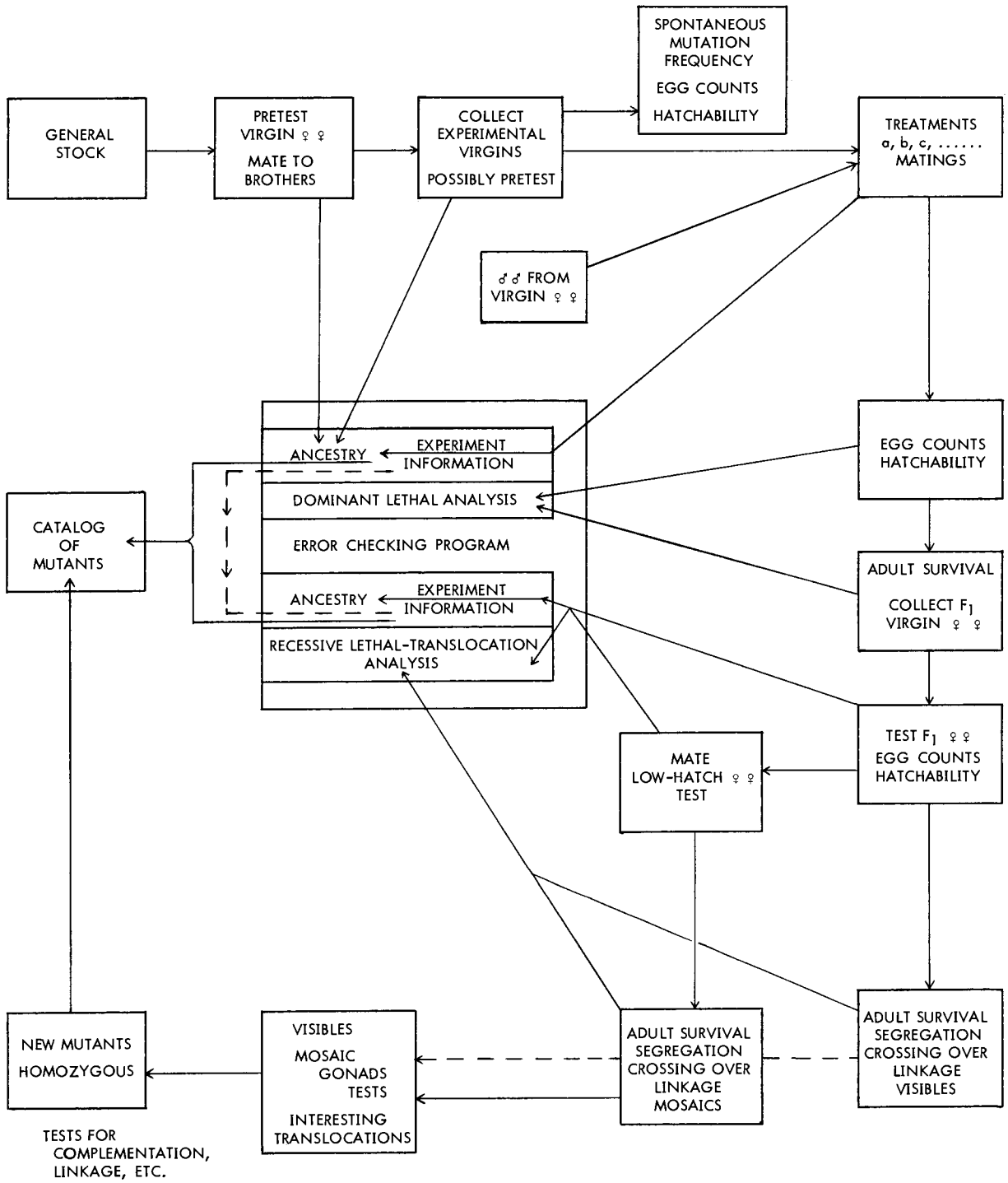
6.2.4.0 If an effect is observed on operation 6.2.3.1, then operation 6.2.3.3 will become operation 6.2.3.2

6.2.5.0 If an effect is observed with operation 6.2.3.2, then operation 6.2.3.7 will become operation 6.2.3.3

Fig. 6.2-1. Each Analysis Requires a Full Complement of Personnel in Order to Complete Necessary Operations.

6.2.6. Appendix — Flow Diagram of the Habrobracon Mutational Experiment

FLOW DIAGRAM: HABROBRACON MUTATION EXPERIMENTS



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