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SYNERGISTIC EFFECT OF ZERO-G  
AND RADIATION ON WHITE BLOOD CELLS  
AND *NEUROSPORA* SPORES

Annual Report  
Period Ending June 30, 1966

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BIOLOGY DIVISION

**SYNERGISTIC EFFECT OF ZERO-G  
AND RADIATION ON WHITE BLOOD CELLS  
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Annual Report  
Period Ending June 30, 1966

This research is carried out at ORNL under NASA order number R-104 Task 4

MARCH 1967

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

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## SUMMARY

This report describes work performed during the fiscal year ending June 30, 1966, on a repetition and extension of the S-4 experiment, entitled "Synergistic Effect of Zero-G and Radiation on White Blood Cells and *Neurospora* Spores." The original S-4 experiment included only human white blood cells as experimental material. It was carried out at the request of the National Aeronautics and Space Administration under an interagency agreement with the U. S. Atomic Energy Commission, and was executed during the Gemini-III manned space flight of March 23, 1965. The experiment consisted of the irradiation of samples of human leukocytes with  $^{32}\text{P}$  beta particles during the orbital phase of the mission and subsequent cytogenetic analysis of the material to determine chromosomal aberration rates. Because an apparent synergism between radiation and some space-flight parameter was observed in the Gemini-III S-4 experiment, it was suggested to the National Aeronautics and Space Administration that the original S-4 experiment be reflown on a subsequent Gemini mission, and further that it be extended to include tests on a second biological material, the bread mold *Neurospora crassa*, in addition to the human leukocyte system used for the original experiment. The augmented S-4 experiment was accepted for the Gemini-XI mission, scheduled for September 1966.

Work on the original S-4 experiment was begun during January 1964. Progress through June 30, 1964, has been described in ORNL-TM-940. Progress through June 30, 1965, including both the execution of the experiment during the Gemini-III mission and the final experimental results, has been described in ORNL-TM-1550. Although essentially the same Gemini-III experimental device was to be used to carry out the Gemini-XI experiment, the anticipated longer duration of the Gemini-XI mission has required that the blood samples be kept refrigerated during most of the flight. Work during fiscal year 1966 has included design, fabrication, and testing of the new hardware required, as well as biological feasibility tests of both the blood and the *Neurospora* portions of the experiment. Two complete mockup experiments were also carried out, as far as possible under the same conditions, and using the same hardware, equipment, and facilities as anticipated for the Gemini-XI experiment. In addition, a ground duplication of the Gemini-III S-4 experiment was done, in which the same timing, etc., were used as during the mission, but in which the "flight" device was subjected to an approximation of the Gemini-III mission vibration and acceleration profiles on the ground.



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## I. INTRODUCTION

ORNL-TM-940 and ORNL-TM-1550, which report work done on the S-4 experiment project through June 30, 1965, include descriptions of the design of the experiment; the development, qualification, and final form of the experimental hardware; the actual execution of the experiment during the Gemini-III mission; and the experimental results. Following the completion of the Gemini-III S-4 experiment the ORNL Biology Division suggested to the National Aeronautics and Space Administration that the experiment be reflown on a subsequent Gemini mission in order to attempt to confirm the apparent synergism between radiation and some space-flight parameter. The Division also suggested that ground experiments be undertaken to attempt to establish whether the spacecraft vibration and acceleration profile might be the space-flight parameter responsible for the effect. The repeat S-4 experiment was accepted for the Gemini-XI mission, and work on the project was begun in October 1965. Subsequently, after testing had established feasibility, the ORNL Biology Division requested that the Gemini-XI S-4 experiment be augmented by the addition of a second S-4 experimental device in which spores of the bread mold *Neurospora crassa* would be the biological material irradiated instead of human white blood cells. This extension of the S-4 experiment was also approved for the Gemini-XI mission.

Both the blood and the *Neurospora* portions of the Gemini-XI S-4 experiment utilize the same experimental-device design used for the Gemini-III experiment with little modification. The blood samples, however, are perishable enough so that it was necessary to provide some means of refrigerating the blood experimental device during most of the Gemini-XI mission's planned three-day duration. The new flight hardware was designed and fabricated by the Oak Ridge Y-12 Plant. Qualification testing and documentation were completed during fiscal year 1966, as was fabrication of all flight hardware parts and assemblies.

The long duration planned for the Gemini mission required extensive biological feasibility testing for the blood portion of the experiment. Feasibility studies were also required for the *Neurospora* portion of the experiment. This feasibility testing and the two complete mockups in which both the blood and the *Neurospora* experiments were carried out exactly as anticipated for the Gemini-XI mission were successfully completed during fiscal year 1966. Finally, it was also possible to execute a complete Gemini-III vibration and acceleration experiment during fiscal year 1966.

## II. ORGANIZATION

As was the case for the Gemini-III S-4 experiment, development of the S-4 experiment for the Gemini-XI mission has required the close cooperation of several different Oak Ridge organizations. The design, fabrication, testing, and documentation of the experimental hardware and development of the required supporting equipment have been the responsibility of the Oak Ridge Y-12 Plant. Fabrication of the isotopic beta-radiation sources is the responsibility of the Isotopes Division of the Oak Ridge National Laboratory. Experimental design, biology, and dosimetry are the responsibility of the Biology Division of the Oak Ridge National Laboratory. Statistical analysis is done by the Mathematics Division of the Oak Ridge National Laboratory. The individuals directly responsible for the various phases of the project are as follows:

Biology:	
Human leukocytes:	M. A Bender and P. C. Gooch, Biology Division, ORNL
<i>Neurospora</i> :	F. J. de Serres, Biology Division, ORNL
Isotope sources:	F. N. Case, Isotopes Division, ORNL
Physical design, fabrication, and testing:	H. F. Smith, Jr., Oak Ridge Y-12 Plant
Mechanical:	W. T. Smith, Jr.
Testing:	F. W. Henson
Instrumentation:	R. C. Kinnamon
Radiological physics:	S. Kondo, Department of Fundamental Radiology, Faculty of Medicine, Osaka University, Osaka, Japan
Statistical analysis:	M. A. Kastenbaum, Mathematics Division, ORNL

## III. EXPERIMENTAL DESIGN

### A. Blood

The experimental design of the human leukocyte portion of the Gemini-XI S-4 experiment remains basically unchanged from that used for Gemini-III because the objective is to obtain, as nearly as possible, an exact repetition. Those changes which have been made were made necessary by the planned three-day duration of the Gemini-XI mission. Feasibility tests showed that refrigeration of the blood samples during most of the flight would be required if the samples were to have a reasonable chance of producing adequate cytological preparations after recovery. A nominal refrigeration temperature of 4°C was selected.

It was decided that the postirradiation weightless period should be kept about the same as that for the Gemini-III experiment, that is, about  $3\frac{1}{2}$  hr. To reduce the total  $^{32}\text{P}$  activity of the experimental device, and thus the amount of stray radiation in the spacecraft, it was decided to increase the duration of the exposure of the blood samples from 20 min to 1 hr. Experiments had shown that this decrease in dose rate would have no effect on aberration yield. To have the blood samples at approximately the same temperature during and after irradiation as for the Gemini-III experiment, the refrigeration of the Gemini-XI blood samples will be stopped approximately 1 hr before the irradiation begins.

The experimental material will again consist of a series of ten blood samples, five from each of the same two donors used for the Gemini-III experiment. Four samples from each of the donors will be irradiated with doses of beta rays ranging up to about 250 rads. One sample from each donor will not be irradiated with the others and will serve as the control. As before, two complete experimental devices will be constructed, one for the spacecraft and the other to serve as a duplicate "ground control."

The blood samples will be obtained approximately 9 hr before the spacecraft is launched. The flight experimental device will be installed in its refrigerated bracket aboard the spacecraft about  $2\frac{1}{2}$  hr before launch and the refrigeration started immediately. About 65 hr after launch the refrigerator will be turned off and the blood device allowed to warm up. The irradiation will be started about 1 hr later and stopped approximately 1 hr after that.

### B. *Neurospora*

The bread mold *Neurospora crassa* was chosen as a second biological material to augment the S-4 experiment for several reasons. *Neurospora* has long been the object of extensive research, and its genetics are consequently extremely well known. The ORNL Biology Division has an extensive capability in *Neurospora* genetics with a large group headed by F. J. de Serres working in the area. Both one- and two-hit events can be detected in *Neurospora*, and both gene mutation and survival can be measured. Thus not only may the same sorts of biological end points used in the blood experiment be studied in a second organism, but other end points may be studied as well. Finally, *Neurospora* offers the advantage that the asexual spores may be treated and handled in large numbers and will remain viable for fairly long periods without refrigeration.

As is the case for most nonmammalian materials, radiation doses in the kilorad range are commonly used for radiobiological experiments with *Neurospora*. Since the spores are in a "dormant" state, however, these doses may be delivered over fairly long times without causing any difficulty in interpreting the results of the experiment. The dose rates delivered to the sample chambers in the experimental device originally designed for the blood experiment are sufficiently great to give the required doses in a reasonable length of time.

It was decided to use two different *Neurospora* spore samples for the experiment: a suspension of spores in an aqueous medium, and dry spores deposited in a layer on a Millipore filter membrane.

The spore suspension will occupy one of the sample chambers in each sample holder in exactly the same manner as a whole blood sample. The geometry is thus the same, and the dosimetry calculations made for the blood experiment need only be corrected for the slight difference in density between the two liquids. The dry spore samples, on the other hand, take the form of a very thin disk supported equidistant between the windows of the sample chamber. The geometry is thus quite different from that of the liquid samples, and there is very little difference in dose between the surface and the middle of the sample layer. The fluoroglass dosimeters in the chamber plug screws used for monitoring doses in the liquid samples cannot be used for the dry spore layers. Instead, thin thermoluminescent dosimeter disks of Teflon loaded with lithium fluoride (ConRad) will be incorporated in the dry spore sample layers.

With the exception of the different dry spore layer geometry and dosimeters, then, the *Neurospora* experiment is essentially the same as the blood experiment. The same experimental device will be used, and the  $^{32}\text{P}$  radiation sources will have the same total activity as those used for the blood experiment device. The *Neurospora* experimental device will not be refrigerated, however. In order to duplicate the blood experiment as much as possible, the irradiation will be terminated at the same time as for the blood experiment. The irradiation will have to be started much earlier, though, to achieve the total doses required for the *Neurospora* samples.

The experimental material will thus consist of a series of ten *Neurospora* samples: five as suspensions and five as dry spore layers. Four of the samples of each type will be irradiated with doses of beta rays ranging up to about 15 kilorads. One sample of each type will not be deliberately irradiated and will serve as the control. As in the case of the blood experiment, two complete experimental devices will be constructed, one for the spacecraft and the other to remain at the launch site for the duplicate ground control experiment.

The *Neurospora* samples will be prepared at Oak Ridge and carried to the launch site a few days before launch. The flight experimental device will be installed in the spacecraft about 6 hr before launch. At about the midpoint of the mission, the irradiation of the samples will be initiated. The irradiation will be terminated about 67 hr after launch, simultaneously with the termination of irradiation of the blood samples.

After recovery of the spacecraft, the flight *Neurospora* device will be opened as quickly as possible and the sample holders placed under refrigeration. Both the flight and the ground control samples will be returned to Oak Ridge as rapidly as possible. All of the spore samples will be placed in culture to measure the levels of survival and forward mutation frequencies in each. A comparison will be made of the dose-effect curves for survival and mutation induction to test for possible differences between the flight and ground control samples.

The *ad-3* mutants recovered will be subcultured and then analyzed further by a series of genetic tests to determine the relative frequencies of mutants due to point mutations, which are one-hit events, or to chromosome deletions, which are two-hit events.

## IV. EXPERIMENTAL HARDWARE

### A. Experimental Device

The experimental devices to be used for the Gemini-XI S-4 experiments differ only in minor detail from those used for the Gemini-III S-4 experiment. The thickness of one side wall of the device housing has been increased from 0.063 to 0.125 in. This extra thickness was necessary because the side of the device must make good thermal contact with the surface of the thermoelectric cooler used to refrigerate it in order to maintain satisfactory heat transfer characteristics. In a vacuum, the sides of the original device housing tended to bow due to the pressure of the air sealed inside the device at 1 atm. Thickening the side reduced the degree of bowing to an acceptable level.

In order to permit use of dry spore sample layers in the *Neurospora* experiment, it was necessary to change the procedure for assembly of the sample chambers somewhat. The design of the sample holders, however, is unchanged. One window of one of the sample chambers for each of the sample holders for the *Neurospora* experiment is simply left off until after insertion of the specimen. It is then attached to the holder with Eastman 910 adhesive. Figure 1 shows the components of the *Neurospora*-type sample holder. The spore layer is deposited on one of the Millipore filter disks (4) with two lithium fluoride-impregnated Teflon thermoluminescent dosimeter disks (5) each 0.005 in. thick (ConRad) incorporated into the layer to monitor radiation dose. The spore layer is covered with a second Millipore filter, and the whole is placed between two disks of polyurethane plastic foam (3) for support. The resulting sandwich is placed in the sample chamber and the window (6) glued down to complete the assembly. The two filling holes are plugged with cotton to maintain sterility while still allowing gas exchange. Sterility must, of course, be maintained during assembly. A cross section through the dry spore sample chamber is depicted in Fig. 2.

The experimental device was flight qualified and successfully flown on Gemini-III. In consequence, no further qualification testing was necessary for the Gemini-XI mission. Sufficient experimental device parts have been fabricated for both mockup experiments and the actual experiments. The flight and backup hardware has been inspected and certified as GFA equipment.

### B. Blood Device Refrigerator and Bracket

Both the refrigerating device in which the blood experimental device is housed during the flight and the metal mounting bracket by means of which the assembly is attached to the spacecraft hatch, and through which heat is transferred from the experimental device to the hatch structure, are new hardware not used for the Gemini-III S-4 experiment.

**Bracket.** — The mount bracket is similar to the bracket furnished on Gemini-III by McDonnell Aircraft Corporation, but some lightening holes have been removed, the mounting hole pattern has been relocated, and the face angle has been changed slightly to provide additional clearance for the refrigerator housing. The Oak Ridge version of the bracket is shown in Fig. 3. Since all of the changes from the original McDonnell design are such as to strengthen the bracket structurally, no flight qualification testing was necessary. Flight and backup brackets have been fabricated, inspected, and accepted as GFA equipment.

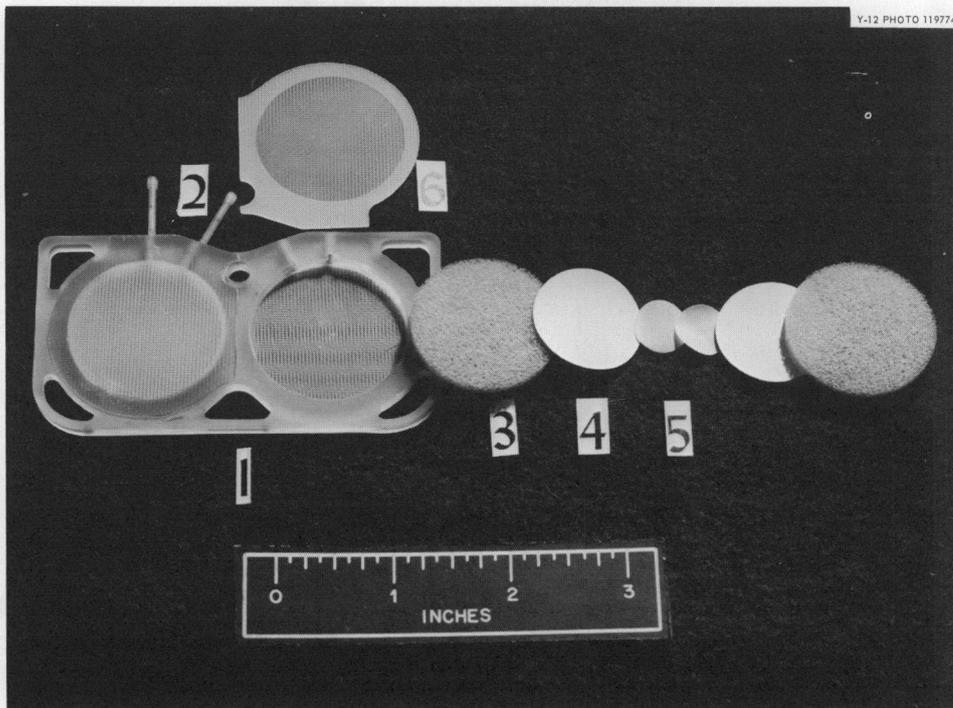


Fig. 1. *Neurospora* Sample Holder Assembly. The holder (1) is fabricated exactly like the blood sample holder except that the window (6) of one of the chambers is left off until final assembly, and dosimeter screws (2) are used only on the liquid sample side. The spore layer is deposited between the Millipore filters (4) with the Teflon-LiF dosimeter disks (5). The whole "sandwich" is centered in the chamber cavity by two disks of polyurethane foam (3).

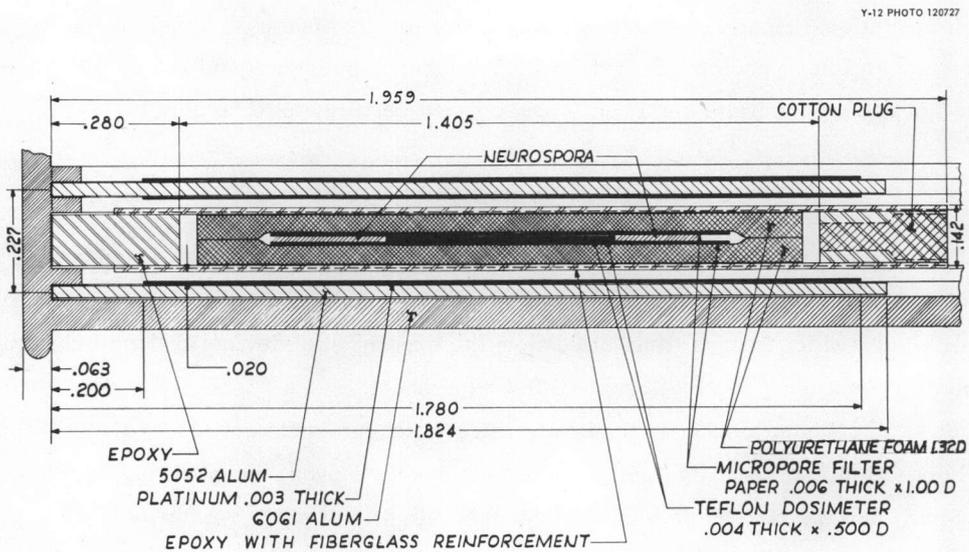


Fig. 2. Drawing of a Cross Section Through a *Neurospora* Dry Spore Sample Chamber, Showing the Structure of the Sample "Sandwich."

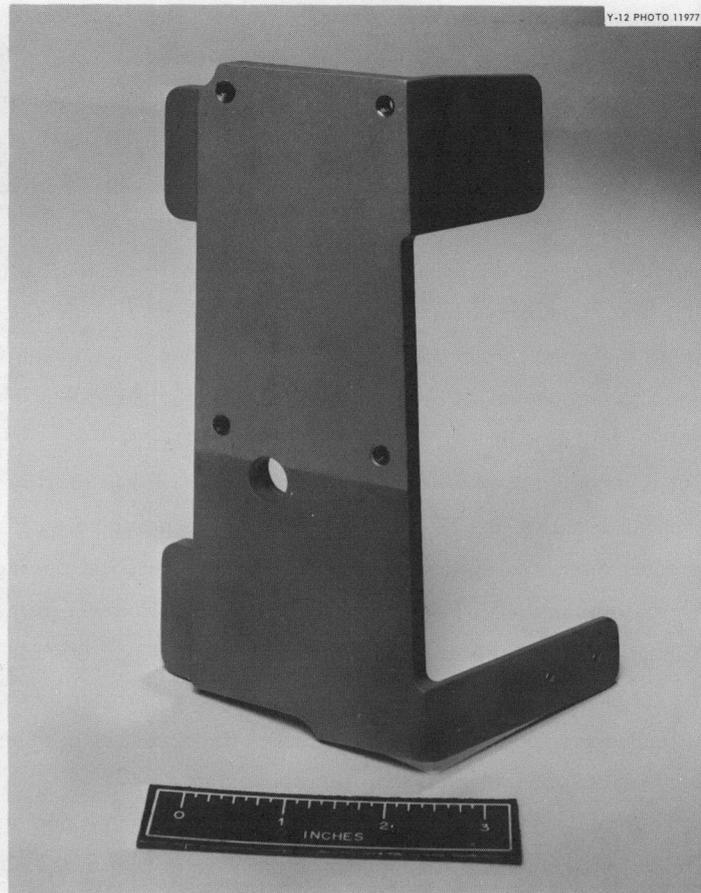


Fig. 3. Torque Box Mounting Bracket for the S-4 Blood Experimental Device.

The bracket is mounted to the inner surface of the left-hand hatch torque box by means of rivets. In order to facilitate heat transfer to the torque box, a silver-loaded silicone heat-transfer grease is applied to the mating surfaces before assembly. The flight bracket has already been installed in the spacecraft by McDonnell Aircraft Corporation.

**Refrigerator.** — A solid-state thermoelectric cooling device seemed the only reasonable means of cooling the blood experimental device during flight. Such a device had in fact been constructed by General Electric Company for the S-3 experiment carried on Gemini-VIII. NASA agreed to make a maximum of 20 w of spacecraft power available for the S-4 experiment from the Agena Control Circuit and also to provide the same temperature telemetry channel and power used for the Gemini-VIII S-3 experiment. A switch has been provided on the pilot's instrument panel for turning the unit on and off.

The thermoelectric cooling unit is basically a low-voltage, high-current device. The spacecraft power available is a nominal 26 v dc. A vibrator-transformer-rectifier circuit would thus have been required to operate any of the commercially available thermoelectric cooling modules. This

sort of design, which was used for the S-3 experiment, presents some serious radio-frequency interference and transient reflection problems. It was therefore decided to have a thermoelectric module developed which could be operated directly from the available spacecraft power. Development work and the fabrication of the final units were done under subcontract by Borg-Warner Research Laboratories. In its final form the device consists of a large number of individual bismuth telluride units arranged in series (Figs. 4 and 5).

**Control and Telemetry Circuits.** — A simple proportioning control circuit was designed to provide temperature regulation. Temperature of the experimental device is sensed by a thermistor in the control circuit, while a second thermistor provides temperature information for the telemetry circuit which was designed to be compatible with the spacecraft PCM telemetry channel provided. A schematic of the circuitry is shown in Fig. 6.

**Housing.** — An aluminum housing was designed with a hinged lid to facilitate last-minute insertion of the experimental device (Figs. 7 and 8). Spaces were provided within the lid and the end of the housing for the electronics, as shown in Fig. 9. The experimental device is completely surrounded by a layer of polyurethane foam insulation except for the area where it is in contact with the copper heat transfer plate of the thermoelectric cooler itself. Good thermal contact with the plate and also with the thermistor bead is assured by the application of a layer of zinc oxide-loaded silicone heat transfer grease to the mating surfaces during assembly. A single plug at the rear of the unit provided electrical interface with the spacecraft.

**Qualification and Testing.** — A qualification test unit was fabricated and subjected to the required tests, including pressure, oxygen atmosphere, temperature, humidity, vibration, acceleration, shock, radio-frequency interference, and transient susceptibility (Figs. 10 and 11). The device met all of the test criteria successfully. An overload qualification shock test was required because of the device's location on the hatch, where it would be subjected to extreme shock in the event that it became necessary to use the crew's ejection seats in a low-altitude abort. The device was subjected to a fast-rise sawtooth pulse of 159.8g for a duration of 66 msec on the Naval Ordnance Laboratory's 21-in. air gun (Fig. 12). Not only did the device meet the criteria that it remain intact and fastened to its mounting bracket, but it also survived the test fully functional and has, in fact, been used in the mockup experiments described below.

A total of six refrigerator units have been fabricated. All have successfully passed functional verification testing. Flight and backup units have been delivered to McDonnell Aircraft Corporation and installed on the Gemini-XI spacecraft. Unfortunately, several of the units have been physically damaged during or after installation in the spacecraft. The damaged units have been repaired in Oak Ridge and returned to McDonnell, however. Figure 13 shows the S-4 blood experiment hardware as it is installed on the spacecraft hatch.

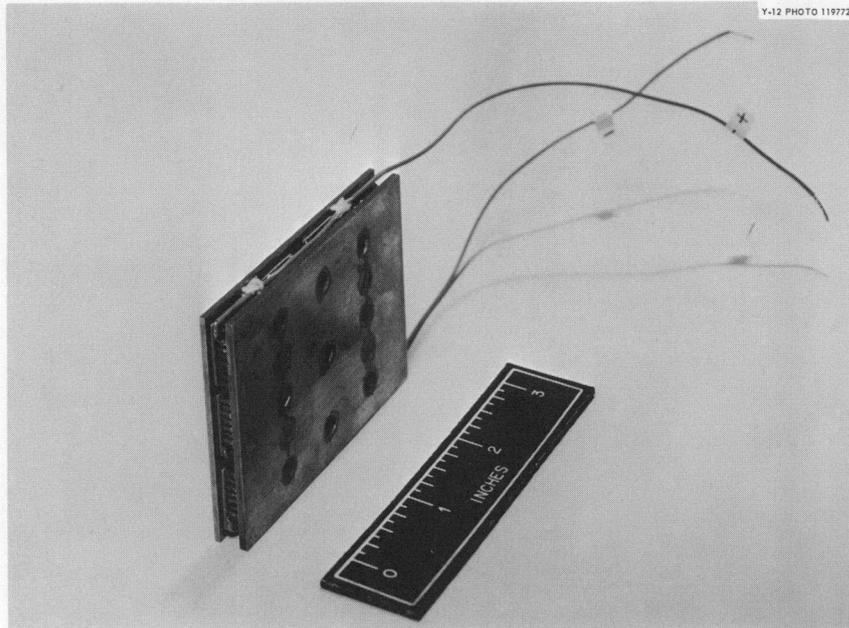


Fig. 4. Special High-Voltage, Low-Current Thermoelectric Cooling Module Developed for the S-4 Experiment Refrigerator.

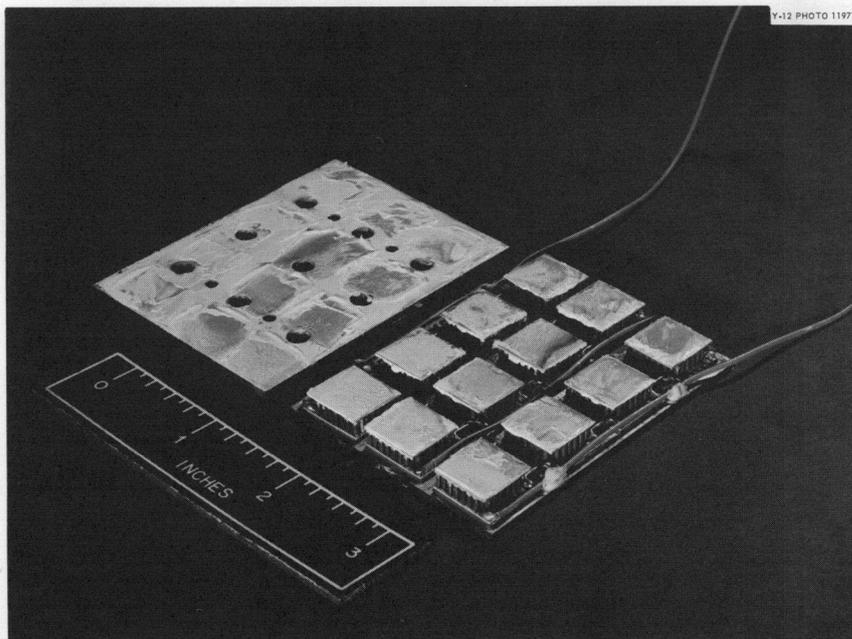
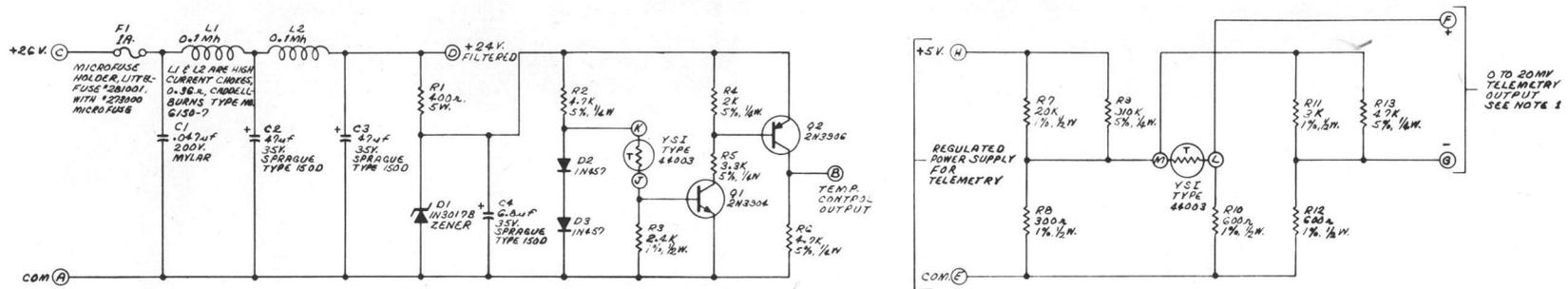
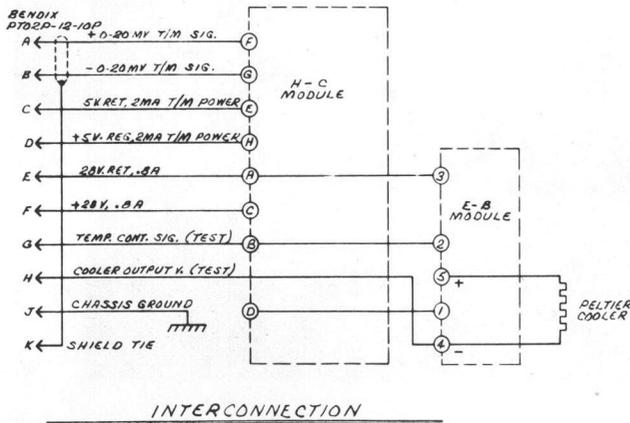


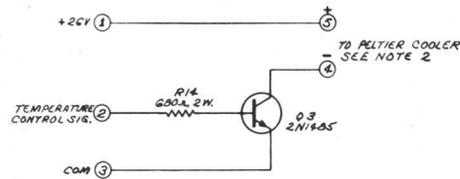
Fig. 5. Thermoelectric Cooling Module. One heat transfer plate removed to show construction. The individual bismuth telluride blocks are visible. The grease visible on the surfaces of the individual arrays is a dielectric zinc-oxide-loaded silicone used for heat transfer.



H-C MODULE



INTERCONNECTION



E-B MODULE

NOTES:

1. TELEMETRY CIRCUIT DESIGNED TO GIVE 0-20 mV OUTPUT FOR +30° TO 100° TEMP RANGE.
2. DO NOT OPERATE E-B MODULE WITH LESS THAN 4.0A BETWEEN TERMINALS 1 & 4 AS IT WILL PROBABLY DESTROY THE TRANSISTOR.

Fig. 6. Schematic Diagram of S-4 Experiment Refrigerator Circuitry.

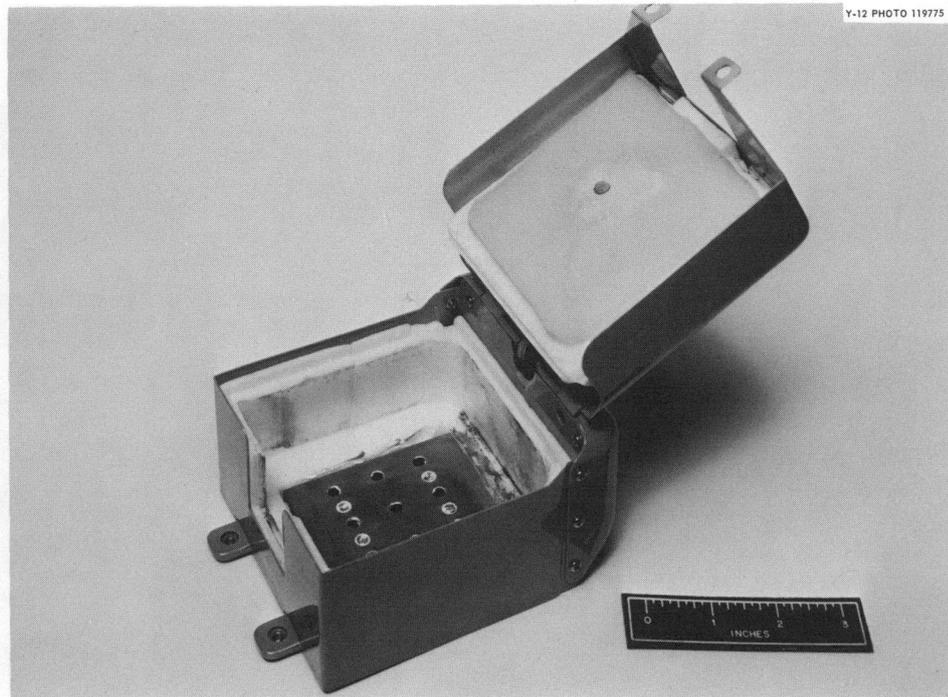


Fig. 7. S-4 Experiment Refrigerator Unit. The heat transfer surface of the thermoelectric cooler itself is visible in the bottom of the device, surrounded by foamed plastic insulation. The electronics are housed in spaces in the end and top of the device. The thermistor bead is visible in the center of the insulation in the open top cover.

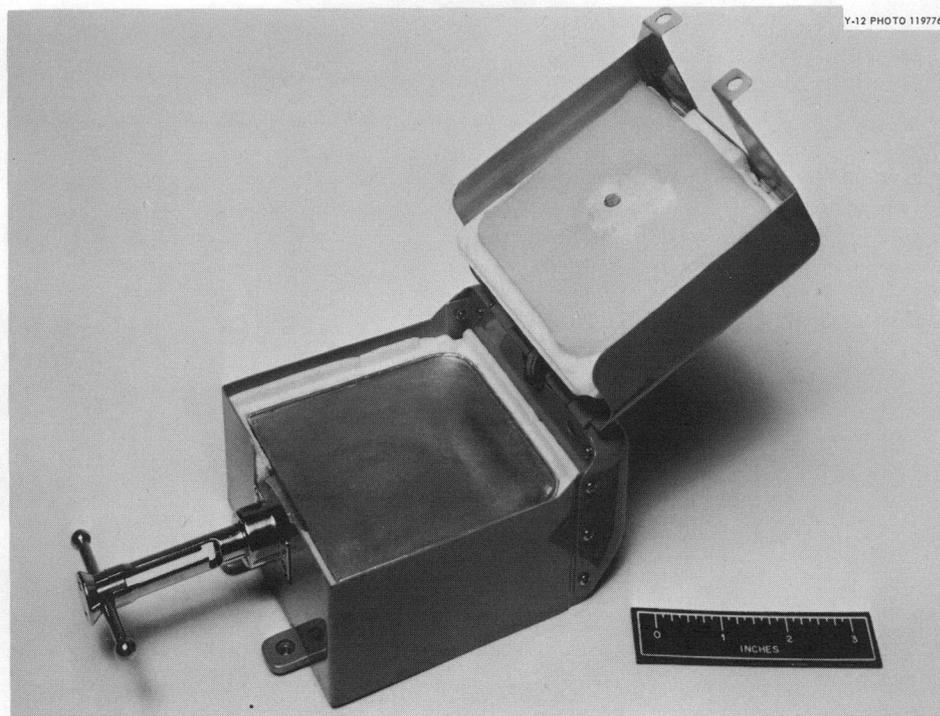


Fig. 8. S-4 Refrigerator Unit Showing the Blood Experimental Device in Place.

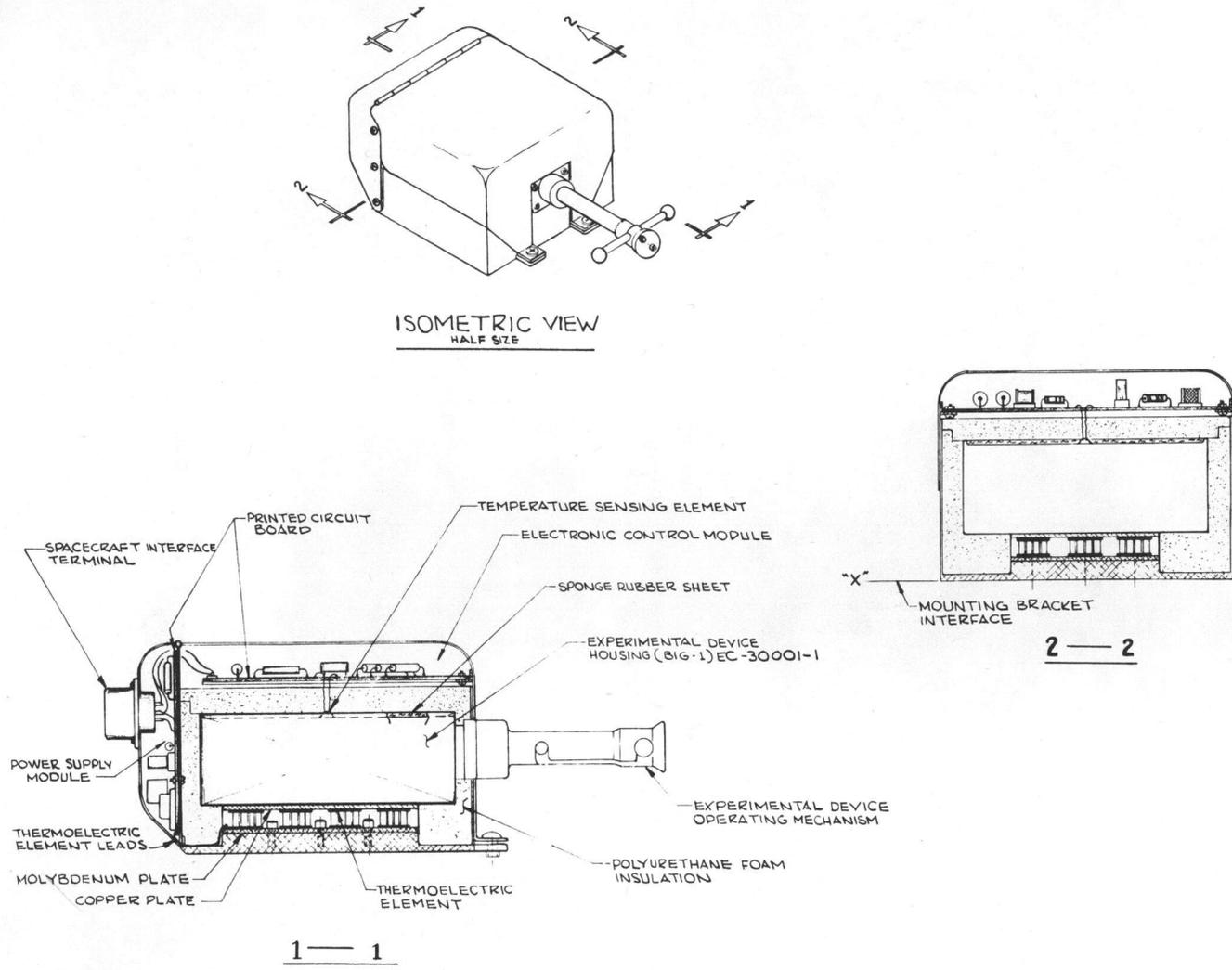


Fig. 9. Drawing of S-4 Refrigerator Unit Showing Location of Circuit Boards and Other Components.



Fig. 10. Vacuum Chamber Setup Used for Qualification Testing of the S-4 Refrigerator. The refrigerator is located under bell jar.

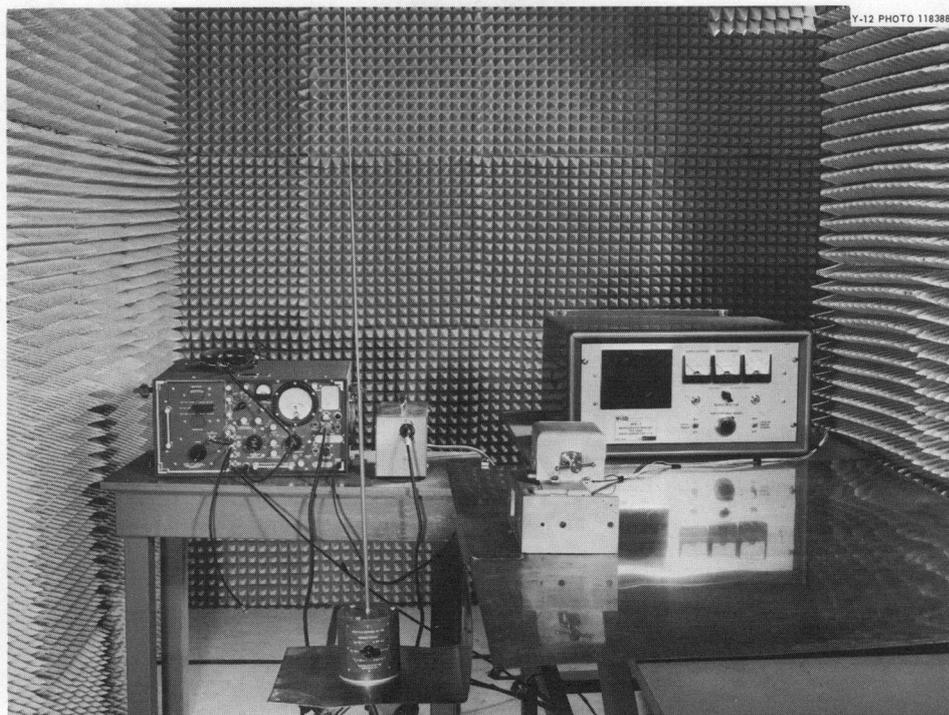


Fig. 11. Radio-Frequency Interference Test Setup During Qualification Tests of the S-4 Refrigerator Unit.

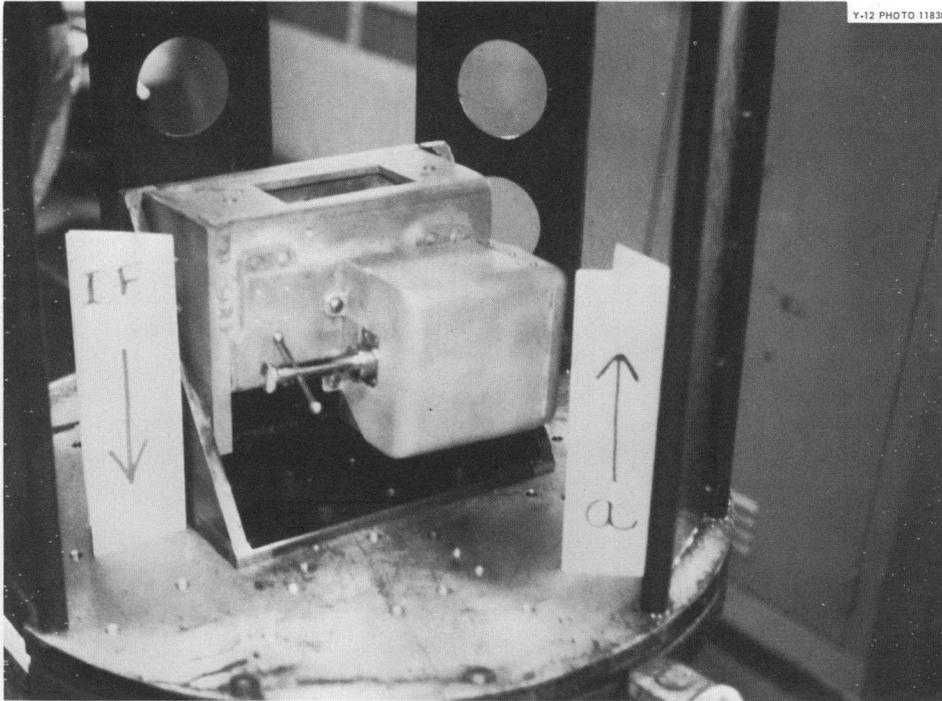


Fig. 12. S-4 Refrigerator Unit in the Naval Ordnance Laboratory's Air Gun Used for Overload Shock Qualification Test. Arrow (a) indicates direction of acceleration.

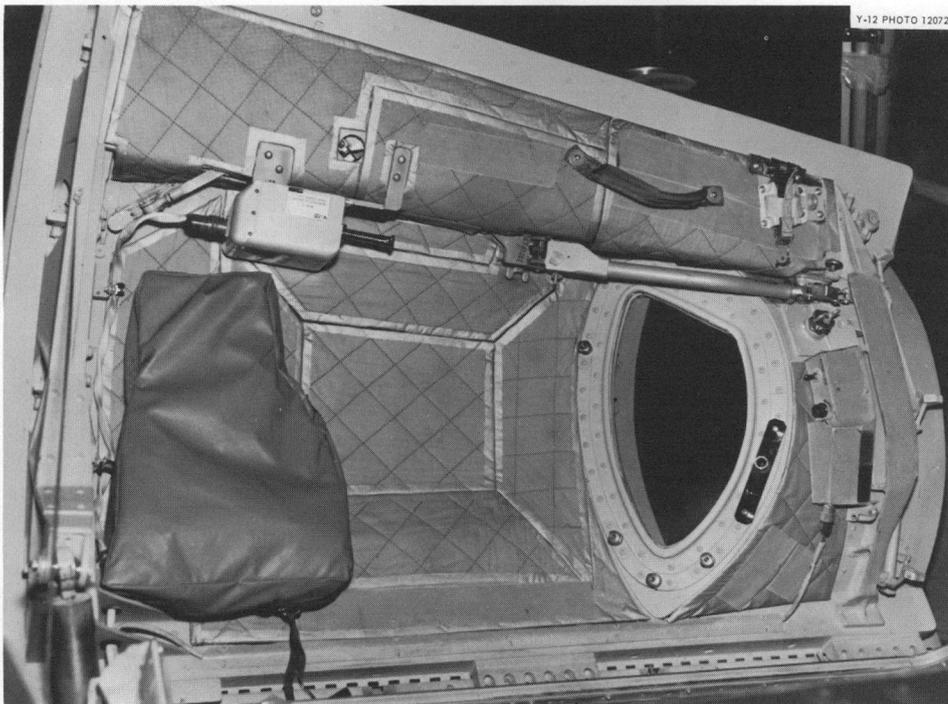


Fig. 13. S-4 Blood Experiment Bracket, Refrigerator, and Experimental Device as Mounted in Position on the Spacecraft Left Hatch.

### C. Supporting Equipment

A test unit has been designed for the S-4 blood experiment refrigerator, and three units have been fabricated (Fig. 14). These units provide a power supply, heat sink, and means of monitoring pertinent refrigerator control and telemetry circuit functions. One unit has been supplied to the McDonnell Aircraft Corporation as aerospace ground equipment for use in preinstallation acceptance testing. A second unit has been installed in the ORNL laboratory-shop trailer, which will be located outside the Mission Support Operations Building on the Merritt Island launch area before and during the Gemini-XI mission. The trailer unit will be used to operate the refrigerator for the ground control portion of the S-4 blood experiment during the mission. The third test unit has been used in Oak Ridge for both testing and mockup experiments.

### D. Instrumentation

Essentially the same instrumentation package will be used for the Gemini-XI S-4 experiments as was used for the Gemini-III experiment. However, because of the Gemini-XI mission's much longer planned duration, several minor changes have been made. These include changing the rate at which the microcoulometer "clocks" run, and choosing new temperature switching points such that all of

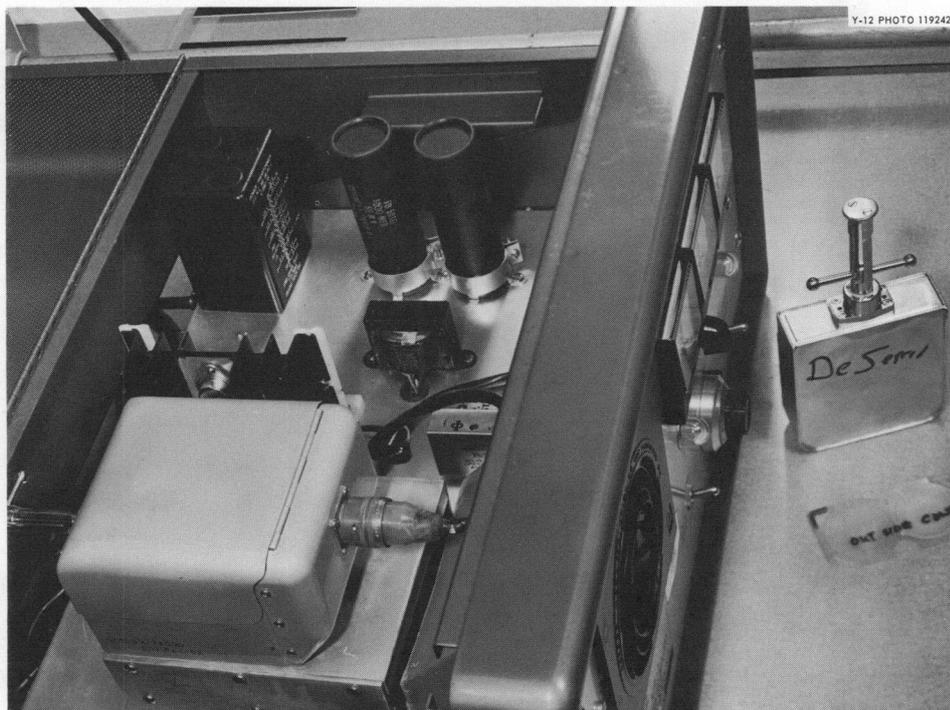


Fig. 14. S-4 Experiment Pre-Installation Acceptance Test Unit. The blood refrigerator is shown in place on the unit's heat sink with the experimental device in place during a mockup experiment. The *Neurospora* experimental device fabricated during this mockup experiment is standing in front of the test unit with the operating handle in the "irradiating" position.

the lamps are off while the device is in the anticipated operating temperature ranges in order to conserve the limited power available from the instruments' batteries. In addition, because the *Neurospora* irradiation period is so long, it was necessary to omit the irradiation-period indicator lamp to avoid using up the battery power before the end of the mission. Instead, a microcoulometer has been substituted in the instrumentation packages to be used for the *Neurospora* experiment to record total irradiation time. The flight and backup instrumentation packages of both types have been fabricated, and testing and inspection have been completed.

## V. BIOLOGICAL TESTS AND EXPERIMENTS

### A. Gemini-III Vibration and Acceleration Mockup

The Gemini-III S-4 results showed a statistically significant difference between the flight and the ground control yields of radiation-induced chromosome deletions. The coefficient of deletion production was approximately twice as great for the flight material. This difference, if real (i.e., if not simply a statistical sampling error), must have been caused by interaction between the radiation administered and some environmental factor associated with the space flight. The three most obvious possibilities are the vibration and accelerations associated with launch and reentry, and "weightlessness" during orbital flight. To check out as many factors as possible on the ground before undertaking another flight experiment, a mockup experiment was performed to test the possibility that either of the first two factors was responsible for the unexpected Gemini-III S-4 result.

Ideally, such an experiment would consist of a duplication of the Gemini-III experiment except that instead of the flight experimental device being flown, it would be subjected to the vibrations and accelerations of the Gemini-III mission on the ground. Unfortunately, it was not possible to do this precisely; no detailed three-axis vibration equipment was available for the experiment, and no vibration data was available from Gemini-III in any case. The vibration and acceleration profiles for the experiment had, then, to be approximations of the Gemini-III profiles.

Three-axis vibration profiles had been taken during the unmanned Gemini-II flight. A tape recording of these profiles was obtained and used for the experiment. A vibration transducer was mounted on the head of a large centrifuge, and connected through slip rings to reproduce the z-axis vibration profile played back through a tape recorder (Fig. 15). The "flight" experimental device was mounted on the vibration head in the mounting bracket supplied by McDonnell Aircraft Corporation for the Gemini-III experiment.

Two experimental devices were made up on approximately the same time schedule as for the actual Gemini-III S-4 experiment. The vibration, accelerations, irradiation, and fixation were done as nearly on the Gemini-III schedule as possible. The doses received by the samples, however, were somewhat different from the Gemini-III samples because of a mixup during assembly which created an array which yielded doses in the ratios 1:1.5:3:3.5, instead of the 1:2:3:4 ratio desired. This was not discovered until after the experiment when the dosimetry was being done. It was not felt that the differences in doses would in any way harm the experiment, especially since the Gemini-III experiment could not be exactly duplicated in any case.

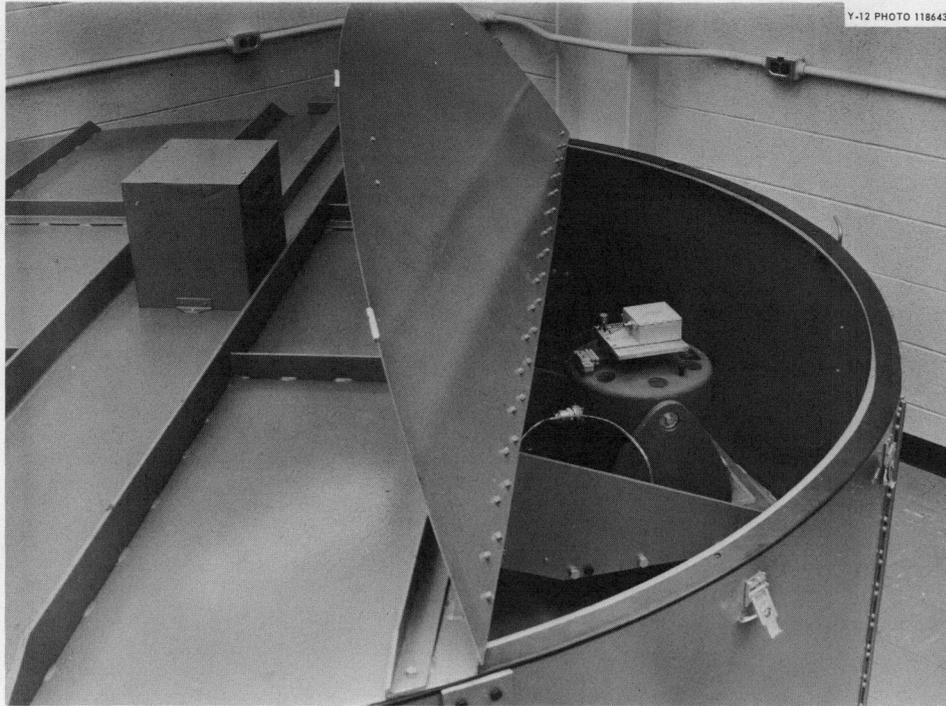


Fig. 15. Experimental Setup Used for the Gemini-III Vibration and Acceleration Experiment. The vibration generator is mounted on the centrifuge used to produce the acceleration profile. The vibration signals are fed to the unit through slip rings on the centrifuge shaft.

The results of the chromosome aberration analyses are shown in Table 1. Least-squares regression analyses were done to determine the coefficients of aberration production. These are given in Table 2. Although the coefficient of deletion production is actually somewhat higher for the material which had undergone the mockup of the Gemini vibration and acceleration profile, the difference from the ground control is not significant.

As already pointed out, the vibration and acceleration test was not an exact duplication of the Gemini-III S-4 experiment. Nevertheless, the failure of the vibrations and accelerations in this test to produce a significant increase in deletion yield argues strongly against these factors being responsible for the Gemini-III result.

### B. Gemini-XI Feasibility Tests

**Blood.** — Although it was already known that the human leukocyte system used for the S-4 experiment could tolerate as much as a day's delay between the time the blood was drawn and the time the cells were put in culture, it was not known whether the delay could be extended enough, even with refrigeration of the samples, for a mission as long as Gemini-XI. A number of simple feasibility tests were therefore undertaken before work was begun in earnest on preparing hardware

Table 1. Results of Experiment S-4 Vibration and Acceleration Test

Sample	Cells Scored	Estimated Dose (rads)	$2n \neq 46$	Chromatid Aberrations	Chromosome Deletions	Ring and Dicentric Chromosomes
Gr <sup>a</sup>	400	4	16	12	10	0
"F1" <sup>b</sup>	400	4	18	13	5	0
Gr	400	62	33	9	22	9
"F1"	400	62	29	11	17	10
Gr	400	83	22	6	21	14
"F1"	400	83	25	9	27	20
Gr	400	168	23	6	41	41
"F1"	400	168	36	16	66	59
Gr	400	196	62	25	62	66
"F1"	400	196	40	17	90	56

<sup>a</sup>Gr = ground control.

<sup>b</sup>"F1" = vibration and acceleration mockup.

Table 2. Coefficients of Aberration Production for S-4 Experiment Vibration and Acceleration Test

	Deletions per Cell per Rad $\times 10^{-4}$	Rings and Dicentrics per Cell per Rad <sup>2</sup> $\times 10^{-6}$
Ground Control	$7.5 \pm 0.9$	$3.8 \pm 0.3$
"Flight"	$9.0 \pm 1.4$	$3.7 \pm 0.7$

for the Gemini-XI S-4 blood experiment. The Gemini-XI mission duration was originally planned as only two days; it was later extended to three days. For this reason, tests were first run for three days and then later had to be redone for four days. The early feasibility tests were done with the blood samples stored in glass bottles, because none of the plastic blood sample holders used for the actual experiment had yet been fabricated. The later tests were made with actual flight-type hardware.

The first tests simply consisted in storing whole blood for three or four days at about 24°C and at about 4°C and then putting them in culture. Mitotic divisions were found in all cultures, but the samples which had been refrigerated yielded better material for cytogenetic analysis.

A second series of tests were done to determine whether the blood samples really needed to be kept as cold as 4°C. The higher the temperature used for storage, of course, the less spacecraft power would be required. Storage temperatures of 4, 10, 15, and 24°C were tested for three days.

As the material stored at 4°C seemed best, this temperature was adopted. No tests were run at four days, because by the time the mission duration was extended the hardware was already committed to the 4°C control point.

An additional series of tests were made to be sure that the blood samples would tolerate the combined insults of the storage required for the mission and the irradiation to which they would be exposed. A single dose (150 r) of x rays was used for these tests, and the samples were refrigerated for three and one-half days, allowed to warm to 24°C, and irradiated. To test the advisability of re-refrigerating the samples after irradiation, some samples were cooled back to 4°C for about 12 hr after the irradiation while others remained at 24°C. All cultures were fixed at four days. The results showed that usable material could be expected from samples both stored at 4°C and irradiated. The re-refrigerated samples were generally poorer than those not re-refrigerated.

To test the possibility that the refrigerated storage of the blood samples might change the leukocyte chromosomes' radiation response, and also to test whether it might be better from this point of view to re-refrigerate the blood samples after irradiation, the material from one of the "storage-plus-irradiation" tests was scored for chromosomal aberrations. In this test three blood samples were obtained from each of two donors and placed in flight-type blood sample holders. All were stored at 4°C for three and one-half days. They were then allowed to warm up to 24°C, and two samples from each donor were given 150 r of x rays. One of the irradiated samples from each of the donors was then returned to 4°C until the cultures were made at four days. The results of the test are shown in Table 3. For comparison, the results expected on the basis of previous x-ray experiments (done in connection with the Gemini-III S-4 experiment) in which no refrigeration was in-

**Table 3. Results of Feasibility Test of the Effect of Storage at 4°C Before or Before and After Irradiation on Chromosome Aberration Yields in Human Leukocytes**

Irradiation was 150 r of x rays. A group of 150 cells was scored from each sample

Sample	Donor	Chromatid Deletions	Chromosome Deletions	Ring and Dicentric Chromosomes
Refrigerated only	A	1	2	3
	B	5	1	0
	Total	6	3	3
Refrigerated and irradiated	A	8	12	23
	B	1	29	23
	Total	9	41	46
Refrigerated, irradiated, and re-refrigerated	A	7	11	17
	B	7	31	33
	Total	14	47	50
Expected with no storage or refrigeration (for 300 cell total)		9	41	41

volved are included. It can be seen that the aberration yields following irradiation are not significantly different from those expected and also that re-refrigeration does not appear to influence aberration yield. As a result of these feasibility tests, plans to re-refrigerate the blood samples following irradiation were dropped.

The full-scale mockup experiments (described in Sect. C) were, of course, the final feasibility tests for the Gemini-XI S-4 experiment. Such tests are required to confirm satisfactory operation of all of the elements which might affect the success of the experiment. Such full-scale tests could not be undertaken, however, before design, fabrication, and qualification of the hardware had been completed.

**Neurospora.** – By the time it was decided to try to augment the S-4 blood experiment by adding the S-4 *Neurospora* experiment, full-scale mockup experiments were about to begin. Consequently, only a few feasibility tests were done before the first mockup. A single "biocompatibility" experiment was done. For this test both suspensions of *Neurospora* spores and dry spore sample sandwiches (described in Sect. IV, A) were placed in flight-type sample holders and stored at 24°C for five days. The samples were then removed from the chambers and placed in culture. Survivals in both the wet and the dry spore samples were found to be adequate for experimental analysis.

A more complete feasibility test was then performed using some of the hardware which had been used for the Gemini-III vibration and acceleration experiment. The activities of the sources in the experimental devices had decayed to the levels to be used for the Gemini-XI experiments two weeks after they were used for the Gemini-III test. A series of *Neurospora*-type sample holders was prepared and the *Neurospora* spores loaded. Since they had already been welded and cut open, the experimental device cases could not be welded shut for this test, but in all other respects the assembly and handling were done as anticipated for the Gemini-XI experiment. Upon culture, the survivals in both the wet and the dry spore samples were found to be adequate. Analysis of the dose-effect curves for survival and forward mutation in the *ad-3* region showed that the planned Gemini-XI experiment was feasible and that it could be expected to yield the scientific information desired.

As is the case for the blood experiment, the final feasibility tests are in fact the complete mockup experiments described below.

### C. Gemini-XI Mockup Experiments

Two complete mockup experiments have been carried out in which the Gemini-XI S-4 experiments were executed in exactly the same way, on the same time schedule, and using the same equipment as anticipated for the actual Gemini-XI mission. The major purpose of the mockup experiments, of course, was the training of the personnel involved. Only one each of the actual blood and *Neurospora* experimental devices was fabricated and used for each mockup; the operations necessary to fabricate the second unit of each type of experimental device were only simulated, in order to reduce the amount of hardware expended. The mockup experiments were both carried to completion,

to provide biological material for analysis, and thus backup information on the chromosome aberration yields and on the *Neurospora* survival and mutation yields to be expected in the actual Gemini-XI S-4 experiments. Analysis of the blood cell preparations from the first of the mockups had been completed by the end of fiscal year 1966. Analysis of the *Neurospora* had not yet been completed, however, and only preliminary results are available.

In the first mockup experiment the irradiation of the blood samples was started 67 hr after the nominal full source strength activity time (12:00 noon on the day of the "launch"), at which time the sources had decayed to about 86% of nominal strength (1.25 microcuries per disk for the most active sources). The blood experiment refrigerator was turned off 1 hr before the start of the irradiation, by which time the temperature of the experimental device had risen to 18.9°C. The irradiation of the blood experiment was terminated 1 hr after it was started. The doses received by the samples, as well as the results of the cytogenetic analysis of the material, are shown in Table 4. The coefficient of aberration production for this experiment was  $(8.2 \pm 1.1) \times 10^{-4}$  deletion per cell per rad, while the coefficient of ring and dicentric production was  $(3.2 \pm 0.6) \times 10^{-6}$  per cell per rad<sup>2</sup>.

The *Neurospora* irradiation was started 1 hr after the simulated launch time and was terminated 64 hr later. Although the survivals and mutation frequencies found on subsequent analysis of the *Neurospora* samples were satisfactory from the technical point of view, it was decided that lower total doses of irradiation would be adequate and that a longer preirradiation "weightless" period would be desirable. It was consequently requested that the *Neurospora* device activation be placed in the Gemini-XI flight plan at a point somewhere between 30 and 40 hr into the mission.

In the second mockup experiment the blood experiment refrigerator was turned off at 62 hr after the imaginary launch. The irradiation of the blood samples was started 63 hr after the "launch," 67 hr after the sources were at their nominal full activity, and terminated 1 hr later. In accordance with the revised plan for the actual Gemini-XI experiment, the *Neurospora* irradiation was started 31 hr after "launch" and terminated at the same time as the irradiation of the blood. Although only preliminary results were available by the end of the fiscal year, the cultures had been successful, and analysis of the material was in progress.

Table 4. Results of Chromosome Aberration Analysis of the First Mockup of the Gemini-XI S-4 Experiment

Cells Scored	Estimated Dose (rads)	$2n \neq 46$	Chromatid Deletions	Chromosome Deletions	Ring and Dicentric Chromosomes
200	8	11	0	2	0
200	73	25	5	18	13
200	137	19	9	32	19
200	202	26	6	31	29
200	266	33	8	40	60

The results of the first two mockup experiments were completely satisfactory from the point of view of functional verification of both the blood and the *Neurospora* portions of the S-4 experiment. However, two additional mockup experiments are planned prior to the actual Gemini-XI experiment. The second of these will actually be carried out at the Merritt Island launch area in connection with the Gemini-XI Simultaneous Launch Demonstration a few days before the scheduled launch.

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