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

Annual Report  
Period Ending June 30, 1967

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ORNL-TM-1995

BIOLOGY DIVISION

SYNERGISTIC EFFECT OF ZERO-G  
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AND NEUROSPORA SPORES

Annual Report  
Period ending June 30, 1967

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

DECEMBER 1967

OAK RIDGE NATIONAL LABORATORY  
Oak Ridge, Tennessee  
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UNION CARBIDE CORPORATION  
for the  
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## SUMMARY

This report describes work performed during the fiscal year ending June 30, 1967, on the Gemini S-4 experiment, "Synergistic Effect of Zero-G and Radiation on White Blood Cells and Neurospora spores." The experiment was carried out at the request of the National Aeronautics and Space Administration under an interagency agreement between that agency and the U. S. Atomic Energy Commission. Biological and radioisotope work was done by the Oak Ridge National Laboratory. Hardware was designed, fabricated, and tested by the Oak Ridge Y-12 Plant. The work during FY 1967 included the execution of the S-4 experiment in connection with the Gemini XI manned spaceflight on September 12-15, 1966.

Work on the S-4 experiment was begun in January, 1964. The first S-4 experiment was carried out in connection with the Gemini III manned spaceflight on March 23, 1965. Progress up to June 30, 1966, has been described in previous annual reports (ORNL-TM-940, ORNL-TM-1550, and ORNL-TM-1715). The scientific results of the Gemini III S-4 experiment have been published in the open scientific literature (Radiation Research 31, 91-111, 1967).

The S-4 experiment consisted of irradiating samples of cells with  $^{32}\text{P}$   $\beta$  particles, both aboard the spacecraft and on the ground, during the orbital phase of the mission. Subsequent analyses allowed comparison of the levels of genetic effect in the in-flight and ground samples. For the Gemini III S-4 experiment, human leukocytes were irradiated, and the chromosomal aberration rates compared. While there was no statistically significant difference in the multiple-break aberration yields from the in-flight and ground portions of the experiment, the yield of single-break aberrations was significantly greater in the in-flight portion of the experiment. The Gemini XI S-4 leukocyte experiment duplicated the Gemini III experiment as closely as possible. In addition, a parallel S-4 experiment was done in which spores of the mold *Neurospora* were irradiated, and survival and mutation were used as biological end points.

The first two months of FY 1967 were devoted to final fabrication, testing, and documentation, of experimental hardware. Equipment and personnel were moved to the launch area several weeks before the flight, and a final mock-up experiment was carried out in connection with pre-launch spacecraft testing. The actual experiment was carried out successfully during the Gemini XI mission on September 12-15, 1966. Post-flight analyses of the recovered flight hardware and instrumentation have been completed; analysis of the experimental material and statistical analysis of the data were virtually complete by the end of FY 1967.

Statistical analysis of the leukocyte data failed to demonstrate any significant difference between the rates of either single- or multiple-break chromosomal aberrations in the in-flight and ground material. The Gemini XI blood experiment thus failed to confirm the apparent synergism seen in the Gemini III experiment.

In the Neurospora experiment, spores were irradiated both as thin films on filters and in aqueous suspension. Analysis of the data for survival and mutation from the spores on filters revealed no significant difference in either end point between the in-flight and ground portions of the experiment. Thus this part of the Neurospora experiment, like the blood experiment, failed to confirm the apparent synergism seen in the Gemini III S-4 experiment. A significant difference was found for survival but not for mutation of the Neurospora spores irradiated in suspension. The significant difference for survival was, however, in the direction of an apparent antagonism, rather than a synergism. This difference between the results for the in-flight and ground suspensions is believed to have been caused by differences in relative anoxia resulting from high cabin temperature, rather than by some factor unique to spaceflight. In any case, it is clear that neither the blood experiment data nor any of the Neurospora experiment data offer any confirmation of the apparent synergism seen in the Gemini III single-break chromosome aberration data.

It is concluded that the difference in the Gemini III S-4 experiment data was probably the result of a statistical sampling error. In any case, even if the effect

suggested by the Gemini III data were in fact "real", it was clearly not the result of a general synergism between radiation and "weightlessness" or some other parameter associated with all orbital spaceflights .

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## I. INTRODUCTION

Work on the S-4 experiment was started on January 15, 1964. Design, fabrication, and test work completed up to June 30, 1964, has been described in ORNL-TM-940. The S-4 experiment was first carried out in connection with the Gemini III manned spaceflight in March, 1965. The results of that experiment were reported in the annual report of the project for fiscal year 1965 (ORNL-TM-1550).

The Gemini III S-4 experiment results indicated that there might actually be a synergism between radiation and some parameter associated with spaceflight, at least for single-break chromosome aberrations in human leukocytes. Therefore, an augmented S-4 experiment was proposed and was accepted for the Gemini XI mission by the National Aeronautics and Space Administration (NASA). Work on the new S-4 experiment was begun in October, 1965. Hardware was again designed, fabricated, and tested by the Oak Ridge Y-12 Plant. Progress to June 30, 1966, included this hardware design, fabrication, testing, and flight qualification, as well as biological feasibility testing, several mock-up experiments, and a vibration and acceleration experiment. These have been described in the annual report of the project for FY 1966 (ORNL-TM-1715).

The period covered by the present report included final hardware fabrication and testing as well as the actual execution of the S-4 experiment in conjunction with the Gemini XI mission on September 12-15, 1966. Following the successful completion of the experiment, analyses were made of the flight hardware and instrumentation as well as of the biological material. A complete dosimetry study was carried out. The biological results have been subjected to statistical analysis. By the end of FY 1967 all work on the project had been completed, except for the detailed genetic analysis of the ad-3 mutants recovered in the Neurospora experiment which is still in progress.

## II. ORGANIZATION

Several different Oak Ridge organizations were responsible for various aspects of the development and execution of the S-4 experiment. Design, fabrication, testing, and documentation of the experimental hardware, as well as development of the supporting equipment, were the responsibility of the Oak Ridge Y-12 Plant. The isotopic radiation sources were fabricated by the Isotopes Division of the Oak Ridge National Laboratory (ORNL). Experimental design, biology, and dosimetry were done in the Biology Division of ORNL. Statistical analyses were performed by the Mathematics Division of ORNL. The persons directly responsible for the various phases of the project were as follows:

**Biology:**

Human leukocytes:	M. A. Bender and P. C. Gooch, Biology Division, ORNL
-------------------	--

Neurospora:	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
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### III. PREPARATION OF THE EXPERIMENT

Most preparations for the experiment had been completed by the beginning of FY 1967. Some final hardware fabrication and qualification remained to be done before the flight, and late repairs, replacements, and tests had to be done because of accidental damage to S-4 experiment hardware installed on the spacecraft at the spacecraft contractor's plant. In addition, a final mock-up experiment was carried out in Oak Ridge prior to the transfer of personnel and equipment to Cape Kennedy for the flight.

#### 1. Hardware Fabrication and Testing

Although most of the flight hardware had been fabricated and accepted as Government Furnished Aerospace Equipment (GFAE) prior to the beginning of FY 1967, the  $^{32}\text{P}$   $\beta$ -radiation plaques had to be prepared shortly before use, because of the relatively short half-life of the radioisotope. Sufficient sets of plaque assemblies, ready for the application of the isotope, were prepared for two final mock-up experiments, as well as for the actual flight. In addition, enough extra plaques were fabricated to allow fresh sources to be prepared in the event of delays in the launch of the spacecraft. Several delays of the start of the Gemini XI mission did actually occur. Because the experimental devices for the flight had already been assembled by the time the mission was called off, each delay caused the loss of source plates as well as other experimental device components. By the time the spacecraft was actually launched after two attempts, the reserve source plates had been prepared at Oak Ridge and shipped to the launch site, and fabrication of a new reserve supply on a "crash" basis had been initiated by the Oak Ridge Y-12 Plant.

During FY 1966, a "flight item" refrigerated bracket for the S-4 blood experiment (plus a flight-qualified backup) had been supplied to the spacecraft contractor for installation in the spacecraft. In spite of the inherent strength and durability of these devices (as witnessed by the ability of the qualification test unit to withstand a fast-rise acceleration pulse of about 160g and not only retain its structural integrity,

but actually to remain fully functional), both the flight and backup units were mechanically damaged by the spacecraft contractor before the spacecraft was shipped to the launch site, and it was necessary to supply new flight-qualified units. Fortunately, extra refrigerated brackets had already been fabricated in case of just such an eventuality, so the damaged units could be replaced quickly.

Final testing of the flight refrigerator bracket was done in the spacecraft after mating with the launch vehicle during the Simultaneous Launch Demonstration (SLD) tests of the spacecraft, its Titan launch vehicle, and the companion Atlas and Agena rockets. During this test, the S-4 telemetry data indicated malfunction of the refrigerator aboard the spacecraft. Upon investigation, however, it was found that the difficulty had been caused by the unauthorized removal of the dummy experimental device from the refrigerated bracket by the white-room crew. This experience enabled us to correct, in advance, what could otherwise have been a serious difficulty during the launch countdown.

## 2. Mock-up Experiments

Two mock-up S-4 experiments had been carried out in Oak Ridge during FY 1966 (ORNL-TM-1715). Scoring of the human blood portion of the second of these for chromosomal aberrations was not finished until early in FY 1967. The results are shown in Table 1. They are in reasonably good agreement with those from the earlier mock-up experiment.

An additional full mock-up experiment was carried out in Oak Ridge prior to the transfer of personnel and equipment to the launch site for the actual flight. The purpose of this mock-up, besides providing additional personnel training and experience, was to obtain additional biological material from which chromosome aberration yields and Neurospora survival and mutation yields could be determined. Only single blood and Neurospora experimental devices were actually assembled and used for this third mock-up. To conserve hardware, the assembly of the "ground" devices was only simulated.

Table 1  
Results of chromosome aberration analysis of the second  
mock-up 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	13	1	4	3
200	73	9	2	5	6
200	137	22	1	16	13
200	202	28	4	38	37
200	266	50	2	56	46

The preparation, assembly, and testing of the devices and the irradiations, opening of devices, and culturing of the material were timed to correspond with the schedule of the then-current flight plan for Gemini XI. Blood was irradiated for 1 hr, starting 67 hr, 40 min after the mock liftoff, and the Neurospora was irradiated for 32 hr, starting 36 hr, 40 min after "liftoff". Only minor changes were made in the Gemini XI flight plan after this mock-up, so the timing was a relatively realistic simulation of the actual mission. Both the blood and the Neurospora material were cultured quite successfully and provided satisfactory material for analysis. Because of the transfer of personnel to the launch site, however, analyses of the material were not completed prior to the actual flight. Since the actual results of the S-4 experiment were both negative and completely consistent, no attempt has been made to finish the analyses of the mock-up material.

#### IV. EXECUTION OF THE EXPERIMENT

##### I. Preflight

Transfer of the laboratory-shop trailer and of personnel to the Kennedy Space Center (KSC) was started on August 21, 1966. The trailer was located just outside the Mission Support Operations (MSO) building at KSC. An additional laboratory facility in the MSO building was occupied (in part) for the filling of the Neurospora sample chambers. This was done outside of the trailer laboratory in order to minimize the chance of contamination of the blood samples by stray Neurospora spores. All facilities and equipment were checked out, and liason with appropriate KSC and Manned Spacecraft Center (MSC) personnel was established. The NASA inspectors who were to oversee the assembly of the experimental devices for the flight were identified and given information and demonstrations to familiarize them with our procedures and thus avoid possible difficulty during the countdown. A communications station was established in the trailer to enable our personnel to maintain contact with various stations at KSC, MSC, and the recovery force. A countdown clock was installed in the trailer.

All personnel, equipment, and sources were on hand for participation in the SLD test, as mentioned above. Neurospora samples were prepared in Oak Ridge and hand-carried to the Cape for the test. Other than the "prelaunch" difficulty with the refrigerator temperature telemetry already described, no major problem was encountered. The actual mission scheduling was followed closely. The S-4 experiment was carried to completion on the basis of the revised flight plan. The resulting blood cultures were quite satisfactory, and yielded adequate material for analysis. "Postflight" experimental procedures with the Neurospora samples were not completed, owing to the short time between the end of the SLD mock-up and the scheduled start of the Gemini XI mission.

Following the completion of the SLD test, two ORNL biologists joined the prime recovery vessel and proceeded to the recovery area. The recovery biologists took

with them sufficient supplies and equipment to set up a laboratory, process the experimental devices, and carry out the necessary culture work following the recovery of the spacecraft. Test blood cultures were made as a test of the facilities and equipment. The results were satisfactory. Liason was established with the NASA and Navy personnel involved in the various phases of the recovery which were of concern to the S-4 experiment. Communications with the launch-area S-4 personnel were established and tested.

On September 2, 1966, sterile peripheral blood leukocyte samples were obtained from the Gemini XI flight crew. Short-term leukocyte cultures were made and fixed after incubation for 63 hr, following treatment with colchicine for 5 hr. The resulting chromosome preparations were satisfactory.

Although a large number of extra flight-qualified experimental device parts were taken to the launch site to provide adequate backup both for accidents in assembly and for last-minute delays in the mission, most of them were consumed by the time the spacecraft was actually launched. The launching was called off on two occasions. Both of these "scrubs" occurred late enough in the launch countdown so that the donors had already been bled, the sample chambers loaded, and the experimental devices assembled before it was known that the launch would not occur. Depending on the exact circumstances, any more than one additional delay might well have left us without enough hardware components to complete the experiment (and quite possibly with insufficient blood in the donors). It appears that an even greater contingency allowance might be wise for any future flight experiments which would require last-minute assembly of flight hardware from nonsalvageable components.

Approximately 10 hr before the actual Gemini XI launch, sterile peripheral blood samples were drawn from the same two donors who had been used for the Gemini III S-4 experiment. The flight and ground experimental devices were assembled and tested, then inspected and accepted for installation in the spacecraft by the NASA inspectors assigned to the experiment. The blood device was installed in the refrigerated bracket on the left-hand spacecraft hatch (Fig. 1) approximately 150 min

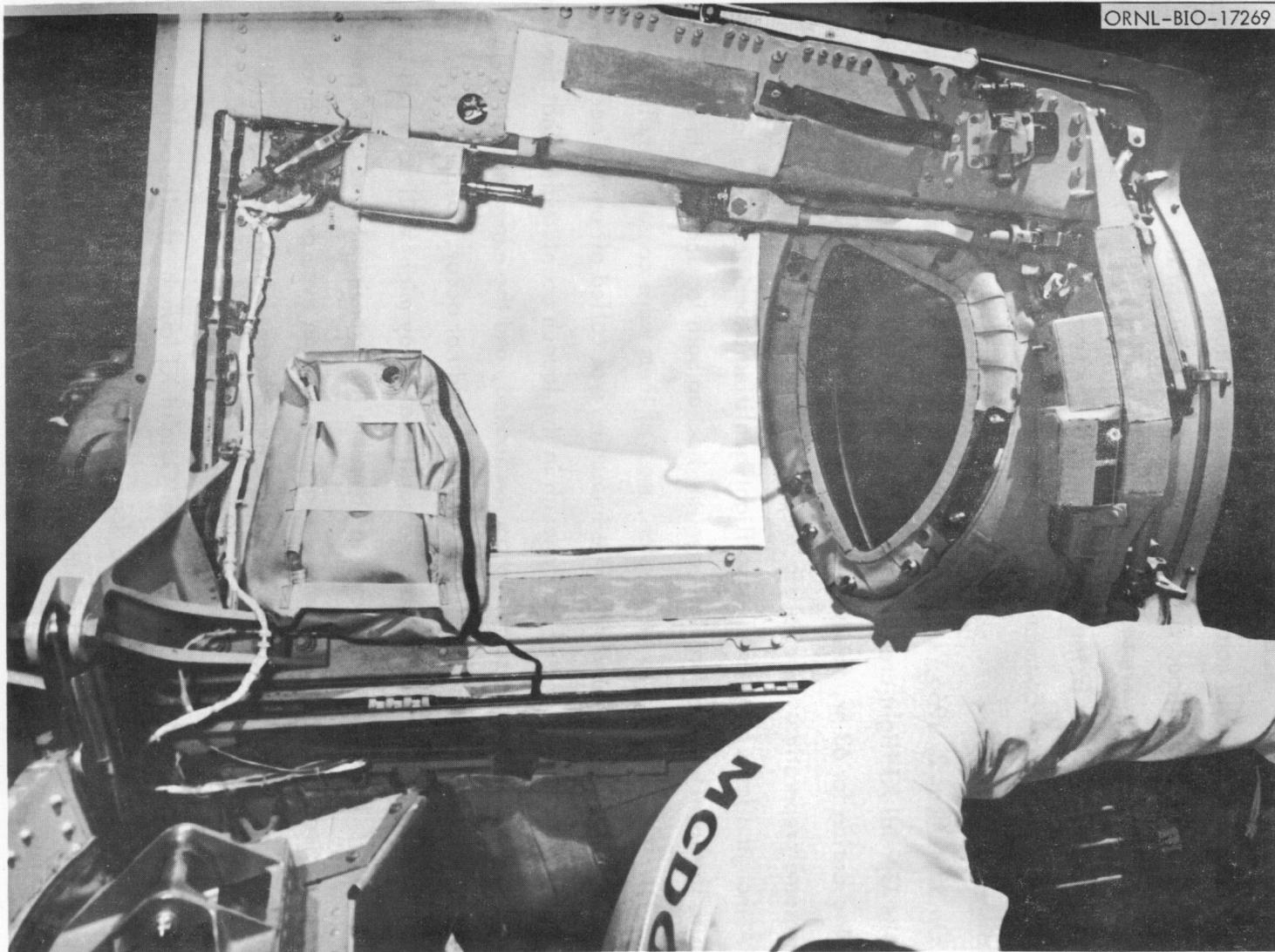


Fig. 1. S-4 blood experiment device mounted in refrigerator bracket on torque box of left-hand hatch of the Gemini XI spacecraft. The electrical connector for power and telemetry may be seen on opposite side of refrigerator from operating handle (photo taken after recovery of the spacecraft).

before launch. The ground control blood experimental device was placed in the duplicate refrigerator located in the laboratory-shop trailer. Both refrigerators were switched on and the temperatures indicated by the telemetry fell rapidly to the normal control range ( $4^{\circ}\text{C} \pm 2^{\circ}$ ), indicating satisfactory operation.

The Neurospora samples were prepared several days in advance of need in Oak Ridge. They consisted both of suspensions of about  $5 \times 10^7$  cells per ml in 0.12% agar solution (used to prevent settling of the spores) and of films of spores collected directly on the surfaces of 25 mm Millipore filter discs. The samples were refrigerated after preparation and hand-carried to KSC. The flight and ground device sample chambers were filled and placed in the experimental devices at about 10 hr and 5 hr, respectively, before the spacecraft launch. Both were refrigerated briefly. The devices were assembled, tested, inspected, and the flight device was accepted as spacecraft equipment. The flight experimental device was installed in the spacecraft on the inboard side of the right-hand footwell (Fig. 2) about 300 min before liftoff. The ground device was kept in the trailer at  $25^{\circ}\text{C}$ .

## 2. Flight

The Gemini XI spacecraft lifted off at 0943 hr E.S.T on September 12, 1966. The spacecraft, and thus the experimental devices, experienced a slowly increasing acceleration along the spacecraft longitudinal (Z) axis reaching a peak of  $5.5\text{g}$  at booster engine cutoff at 00:02:32 GET. The acceleration fell rapidly to about  $1\text{g}$  and then increased slowly again to a peak of  $7.3\text{g}$  at sustainer engine cutoff at 00:05:40 GET. After this the acceleration became essentially zero on all axes.

With the exception of the two Agena primary propulsion system (PPS) burns made in the docked configuration in order to get the spacecraft into and out of its high altitude orbit, only minor accelerations were experienced during the orbital phase of the mission. A large number of orbital maneuvering system (OMS) burns, which produced these accelerations, were made. The TPI burn made early in the mission to initiate the terminal phase of the rendezvous with the Agena produced the largest

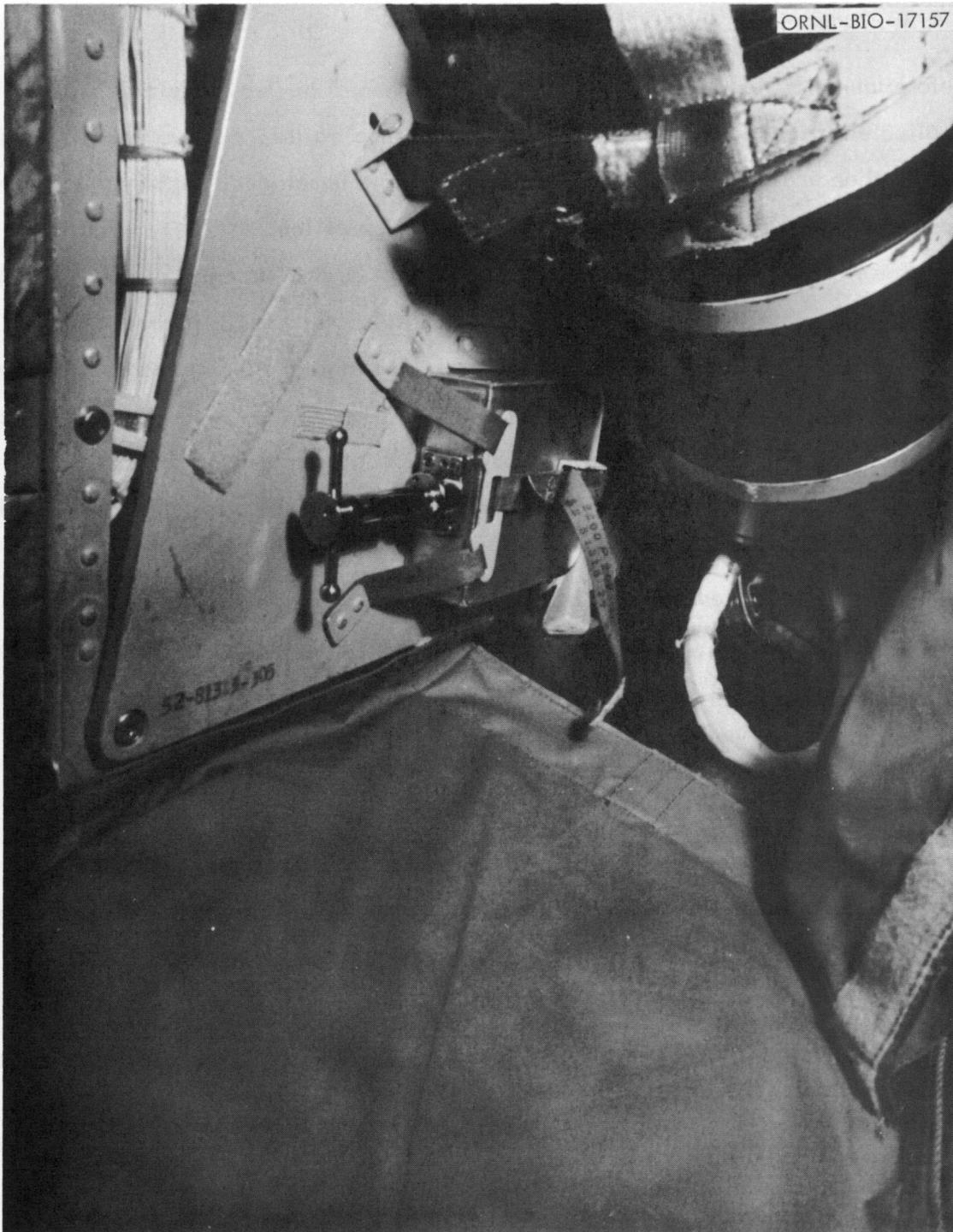


Fig. 2. S-4 Neurospora experiment device mounted on inboard side of right-hand footwell of the Gemini XI spacecraft (photo taken after recovery of the spacecraft).

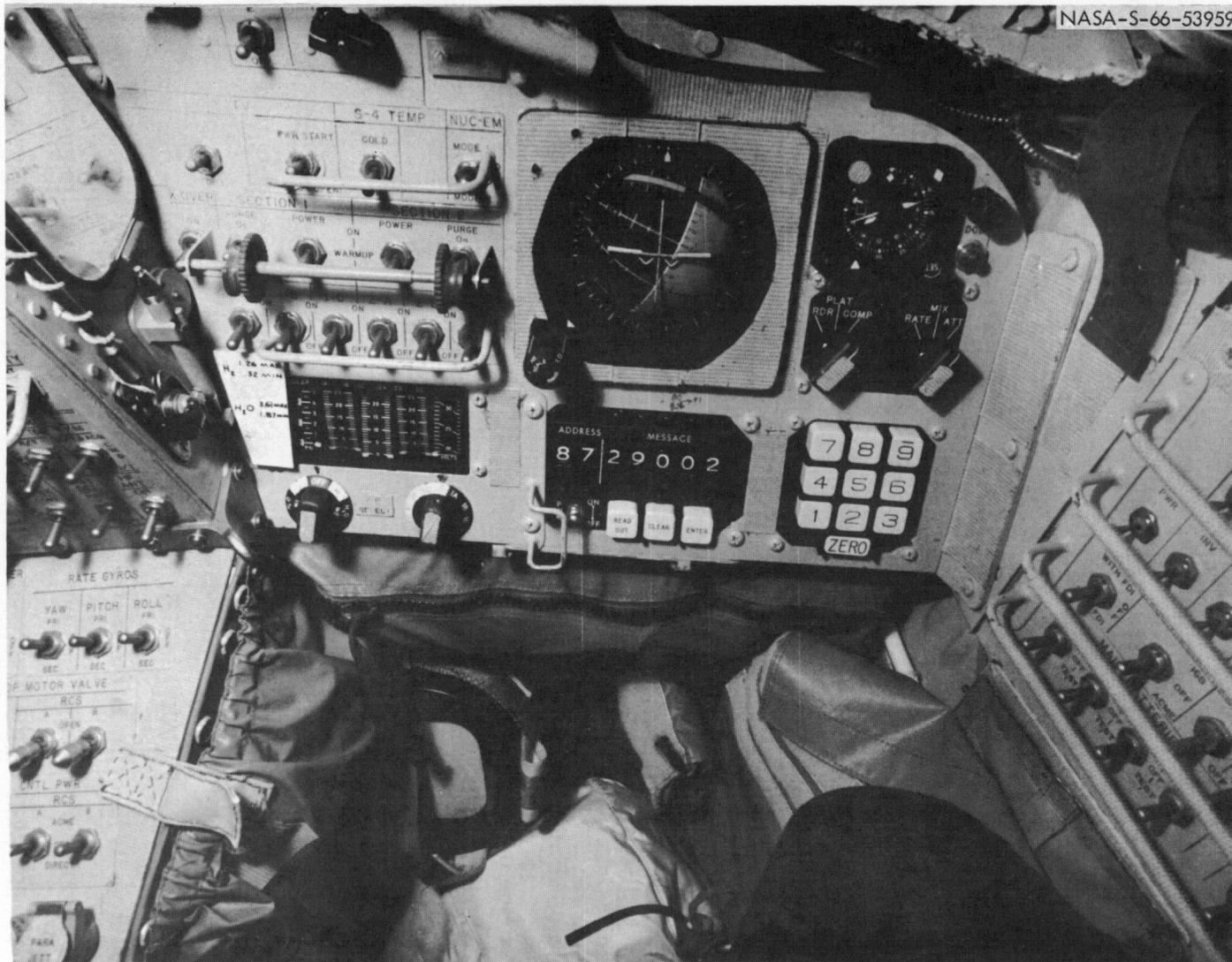
acceleration. This only amounted to a maximum of about 0.04g for a brief period. Most of the other OMS burns were much smaller and of only a few seconds duration. A complete catalog of these burns could not be presented here; in any case it does not appear that they could have exerted a significant influence on the S-4 experiment.

The first (posigrade) PPS burn was initiated at 40:31:25 GET, and produced an acceleration of approximately 1.2g along the spacecraft Z axis for 20 sec. The retrograde PPS burn was initiated at 43:54:05 GET and produced an acceleration of approximately 1.2g along the spacecraft Z axis for 22 sec.

Starting about 14 min after liftoff, the temperature of the experimental device in the refrigerator aboard the spacecraft rose above the normal control range and remained at between 6-10°C through the first 24 hr of the mission. The device temperature fell rapidly back to the normal control range when the spacecraft was depressurized for the first extra-vehicular activity (EVA) of the mission, and with the exception of a brief rise to almost 7°C at about 62 hr GET, operated within this range until the pilot operated the S-4 refrigerator switch (Fig. 3) to turn the refrigerator off at 65:38:00 GET. The ground blood unit remained in the normal control range until its refrigerator was switched off simultaneously with the flight unit. After the refrigerators were turned off, the temperatures of both units rose rapidly to about 25°C, and stayed at about this temperature until they were opened after recovery.

The Neurospora experimental device was stowed in the right-hand footwell during the launch in such a way that the spacecraft Z axis corresponded roughly to a diameter of the sample "discs", and the launch accelerations were thus "along" rather than "through" the samples. After launch, the Neurospora experimental device was removed from its location in the footwell and stowed in a locker, its orientation uncertain, and probably shifting, until just after the irradiation was terminated.

The temperature of the Neurospora experimental device during the mission is not precisely known. The spacecraft cabin temperature telemetry indicates that the temperature was an average of 3-4°C above the 25°C at which the ground Neurospora



NASA-S-66-53959

Fig. 3. Right-hand circuit breaker panel of Gemini XI spacecraft, showing location of S-4 blood experiment refrigerator switch.

experimental device was kept. The temperature sensor for cabin temperature telemetry was located some distance from the position of the Neurospora experimental device during most of the mission, however, and the temperature of the Neurospora device was thus probably somewhat different from that indicated by the cabin temperature sensor. In any case, it seems clear that the flight Neurospora samples were kept at a higher temperature than those on the ground.

The irradiation of the Neurospora samples was begun at 30:09:00 GET. The irradiation of the blood samples was started at 66:43:00 GET. The irradiation of the blood samples was terminated at 67:53:00 GET; the irradiation of the Neurospora samples ended 50 sec later. In all cases the manipulations of the flight and ground experimental devices were simultaneous; each was counted down from the ground in real time. The flight Neurospora samples were "weightless" during all of the irradiation period except for the brief accelerations experienced as a consequence of the two PPS burns. The blood samples were "weightless" through their entire irradiation period. Both blood and Neurospora samples remained in this state following the termination of irradiation until retrofire.

Retrofire was initiated at 70:41:37 GET. The four retro rockets were burned in sequence, producing an acceleration along the spacecraft axis of approximately 0.5g for 22 sec.

Reentry produced a slowly increasing acceleration along the spacecraft Z axis which started at approximately 71:05:10 GET, reaching a peak of 5.7g at about 71:09:21 GET. The acceleration then decreased slowly to slightly over 1g by about 71:12:00 GET. During chute deployment the acceleration on the Z axis briefly reached a peak of over 3g. Splashdown occurred at 71:17:08 GET, and produced peaks of acceleration of 2.3g on the spacecraft X axis and 1.6g on the Z axis.

In summary, after the launch-phase accelerations and vibrations, the in-flight experimental material was essentially "weightless" for the next 70 hr (except for the two Agena PPS burns). This included the irradiation periods, and also the approximately 3.5 hr postirradiation period. The ground material, of course, remained at 1g

throughout the experiment, and experienced no significant vibration. Both the flight and the ground blood samples were refrigerated until 1 hr preirradiation, but were at about 25°C during and following irradiation. The ground *Neurospora* samples were kept at 25°C throughout the experiment, but the flight *Neurospora* samples were probably at a temperature several °C higher throughout most of this period.

### 3. Post-flight

The in-flight experimental devices were removed from the spacecraft shortly after recovery. The blood samples had been removed from both the flight and the ground devices by 73:33:00 GET, and were in culture by 75:14:00 GET. Post-flight peripheral blood leukocyte samples were also obtained from the flight crew and placed in culture on the recovery vessel at the same time. All cultures were fixed after 66 hr of incubation, following treatment with colchicine for 5 hr. The resulting cytological preparations were returned from the launch area and the recovery vessel, and scored for chromosomal aberrations in Oak Ridge.

The samples were taken from the in-flight *Neurospora* device by 73:13:00 GET. The samples were removed from the ground *Neurospora* device by 73:38:00 GET. All of the *Neurospora* samples were immediately refrigerated at 4°C and returned as rapidly as possible to Oak Ridge for culture and analysis.

Genetic analysis of the *Neurospora* spore samples was initiated by preparing suspensions of each for inoculation into an assay growth medium. Inoculation volume was varied so that the total number of survivors expected per test flask (on the basis of the mock-up experiments) would be about  $10^6$  per total flask volume of 10 liters. In the assay medium the heterokaryotic survivors form a tiny white colony about 2 mm in diameter after incubation in the dark for about 7 days at 30°C. Specific-locus mutations of the ad-3A and ad-3B genes cause accumulation of a reddish-purple pigment in the mycelia; such mutations can thus be recognized by the unusual colony color produced.

Five to ten replicate flasks were made from each spore sample. When the flasks were harvested, the total volume of each was measured and the total number of

colonies per flask was determined by counting aliquots. The number of purple colonies was determined by hand-counting in the total flask volume (the number per flask typically varied between 0-500).

The relation between total colony counts and the number of spores inoculated for each of the samples was used to obtain dose-survival curves. The relation between the total number of purple colonies and the total number of colonies per flask was used to obtain the forward-mutation frequencies.

Samples of the ad-3 mutants from each of the samples of spores were retained for a more detailed genetic analysis by complementation techniques. These analyses were in progress during the latter half of FY 1967, but had not yet been completed by the end of the period covered by this report.

## V. EXPERIMENTAL RESULTS

Since it is welded shut, the housing of the S-4 experimental device must be destroyed to some extent by the sawing operation necessary to open it to remove the sample holders. Similarly, both the sealed sample holders and the dosimeters must be damaged in the process of removing the samples of blood or Neurospora spores. A careful visual inspection was made before each device or sample holder was opened, however, in order to detect any leaks or other physical damage. No sign of either leakage or damage was detected. All components from each of the flight and ground experimental devices were placed in special shielded containers and returned to Oak Ridge for further inspection and analysis.

### 1. Instrumentation and Hardware

Upon return to Oak Ridge, the instrumentation packages from all four experimental devices were subjected to detailed analyses. The color film strips from each were removed and developed. They were then scanned with a Beckman spectrophotometer and the readings obtained were translated into temperature and event (irradiation) versus time information. All four instrumentation packages were X-rayed to determine the positions of the microcoulometer gaps. They were then given complete electrical

and operational tests. All results compared favorably with the results of the instrumentation package pre-flight tests, indicating that all four units performed satisfactorily during the experiment.

The records from the flight blood experiment instrumentation package showed that when it was turned on during the final assembly of the experimental device its temperature was above about 22°C, but below 36°C. Its temperature rose to above 36°C briefly during welding. This is confirmed by the spot temperature maximum indicators on the side of the package nearest to the weld. That the blood samples did not become so warm is confirmed by similar indicators on the side of the instrument package farthest from the weld, and nearest the blood sample chambers. The temperature stayed above 22°C until after insertion in the refrigerated bracket on the spacecraft. It fell rapidly in the control range when the refrigerator was turned on and did not rise above 13°C at any time until the refrigerator was switched off prior to the start of the irradiation. Unfortunately, the temperature resolution of these instruments is not sufficient to demonstrate the slight rise in temperature during the first 24 hr of the mission that was indicated by the telemetry data from the spacecraft. At the time the refrigerator was switched off, the temperature of the instrumentation package rose rapidly to above 22°C and stayed above 22°C but below 32°C until the instrument was turned off after the experimental device was opened. The irradiation marker indicates that the irradiation started about 1 hr after the refrigerator was switched off, and that it lasted for approximately 1 hr.

The records from the instrumentation package for the ground blood experiment showed that the temperature was below 36°C when it was turned on during final assembly of the experimental device, and that it remained below 38°C prior to welding. The temperature increased to above 38°C briefly during the welding operation. Spot temperature maximum indicators confirm this rise in temperature, but also indicate that the blood sample temperatures did not go so high. The temperature of the instrumentation package fell rapidly to the refrigerator control range when the refrigerator was started, and did not rise above 9°C until the refrigerator was turned

off again. It then rose rapidly to above 22°C and remained above 22°C but below 35°C until the device was turned off after the experimental device housing was opened. The irradiation marker indicated that the irradiation was started about 1 hr after the temperature started to rise, and that the exposure lasted approximately 1 hr.

Since no temperature extremes were experienced by either the flight or the ground Neurospora experimental devices, the instrumentation package temperature records contain little information other than a confirmation that no temperature extremes were in fact experienced. The temperature for the ground unit rose to above 39°C briefly during the welding operation, but the spot temperature maximum indicators show that the Neurospora samples themselves did not become so warm. The temperature of the flight device stayed below 42°C during welding, but the switch points of the instrument were not such as to make it possible to tell how much lower the temperature maximum during welding actually was. The irradiation-period timers in both packages indicate a total irradiation time of about 38 hr.

Attempts were made both during and after the mission to determine the cause of the slightly high temperature of the flight blood device indicated by the telemetry during the first 24 hr of the mission. The command pilot was able to confirm that the refrigerator was in fact cooling the experimental device; the operating handle was cool to the touch and was "sweating". He also reported that the hatch structure was warm to the touch, indicating that the refrigerator was probably working to capacity, but was not able to keep the temperature in the design range because the heat sink was at a higher temperature than it had been designed for. When the spacecraft was recovered, the thermally conductive grease layers between the experimental device and both the thermoelectric cooler and the temperature sensor were inspected carefully as the experimental device was removed (Figs. 4 and 5). The grease layers were intact, and no clue to the cause of the high temperature was found. After the flight, the flight refrigerator was removed from the spacecraft and returned to Oak Ridge, where it was given thorough electrical and functional tests. No defect or malfunction was discovered. It was concluded that high hatch temperature was indeed the cause of the

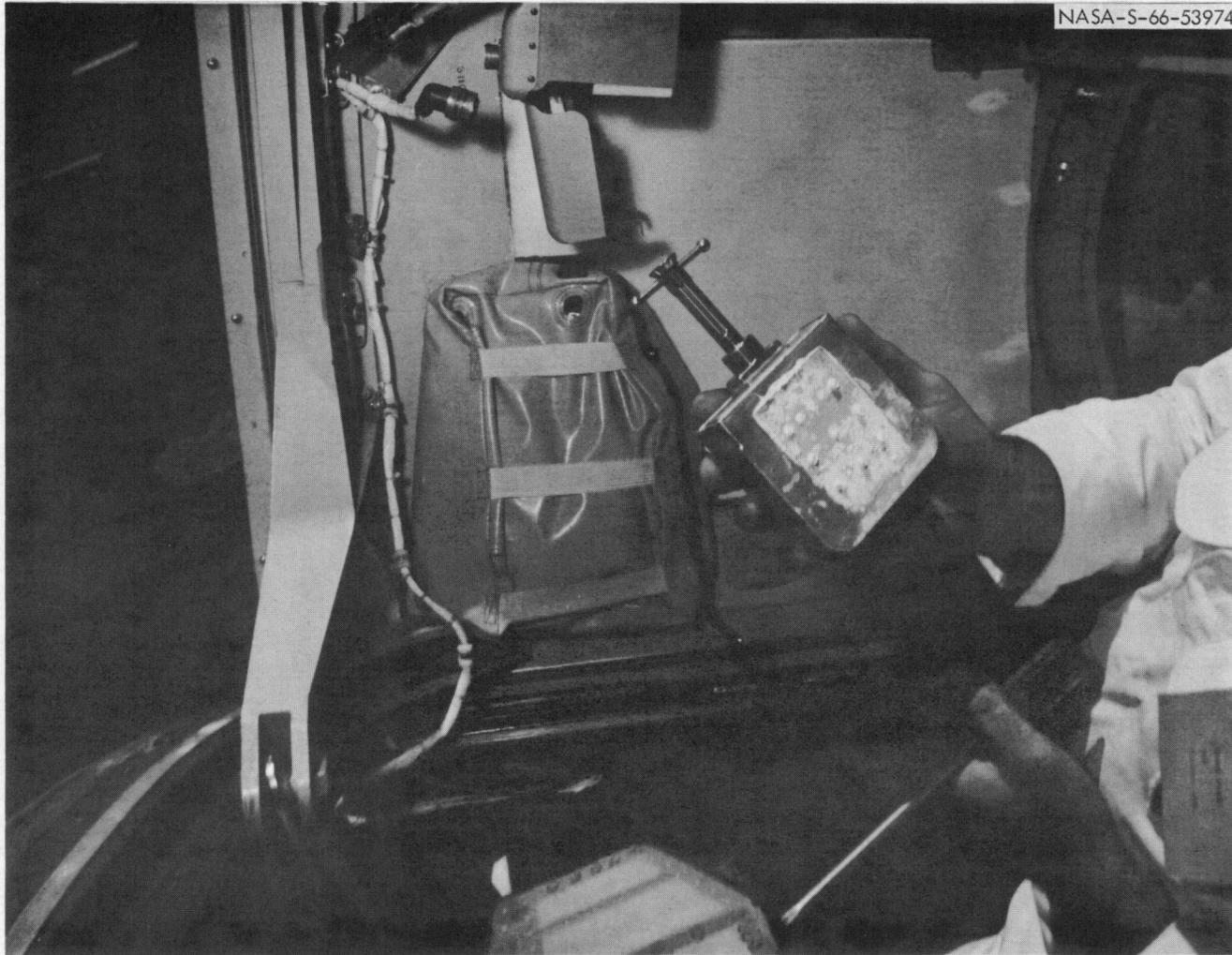


Fig . 4. S-4 blood experimental device as it was removed from the refrigerator bracket shortly after recovery of the Gemini XI spacecraft. The thermally conductive grease layer used to promote heat exchange from the device to the thermoelectric cooler is visible on the side of the device .

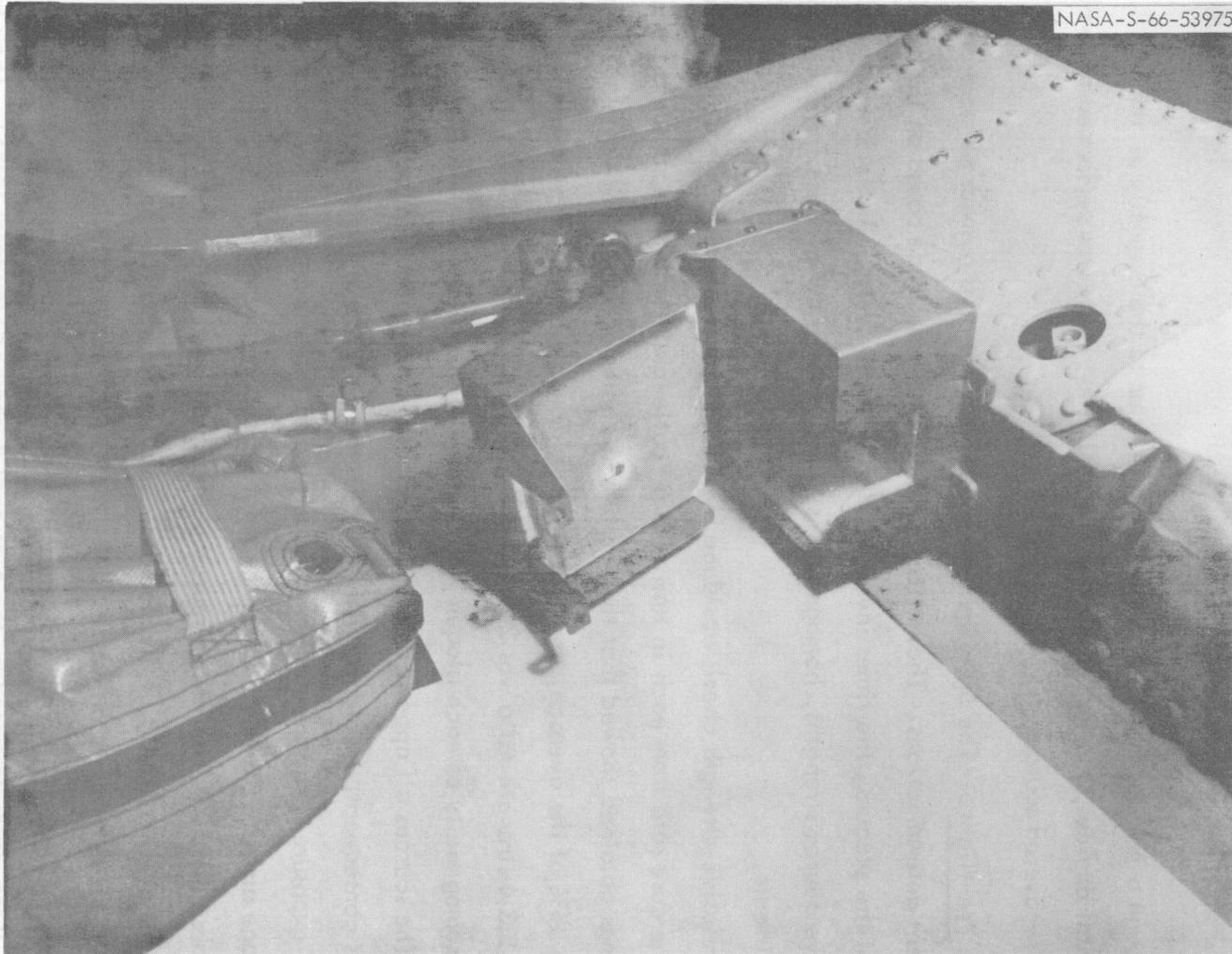


Fig. 5. Interior of S-4 blood experiment refrigerated bracket, showing the copper surface of the thermoelectric cooler inside the bracket, and the temperature sensor inside the hinged cover (photo taken just after removal of the experimental device).

unit's failure to stay in the control temperature range during the early part of the mission.

## 2. Dosimetry

Although a few dosimetry checks were made prior to the assembly of the experimental devices, the largest part of the dosimetric study was performed post-flight, using the recovered sources.

2.1. Pre-flight. — The pre-flight dosimetry depends on a number of more or less independent determinations. Theoretical calculations were made based on the nominal activity of the plaques, the times involved, the geometry, etc. A check was made on the relative-source strengths, homogeneities, etc., before the plaques were used for the experiments.

The relative-strength check was done by placing each plaque in a special jig and reading the dose rate from each in turn with a "cutie-pie" meter in a standard position. The readings obtained showed that the relative strengths of the individual plaques were all within  $\pm 4\%$  of the average, and that the maximum deviation of the averages from a strict 1:2:3:4 strength ratio was 2%.

The homogeneity of each plaque was checked by exposing Polaroid films to each source. The sources all appeared to be acceptably homogeneous, except for one, which was consequently replaced with an acceptable source of the proper strength. Since the exposure times were adjusted according to the source strength, the density of the image also gave a rough check on relative-source strength.

The absolute activities of the source plaques had been determined by direct measurement of the activity of the stock  $^{32}\text{P}$  solution from which the sources were prepared. This determination was made by the ORNL Isotopes Division by means of an end-window, gas-filled  $\beta$  proportional counter which had been calibrated indirectly against  $^{32}\text{P}$  standards prepared by the National Bureau of Standards (NBS). They had their nominal full activities at 1200 hr E.S.T. on September 11, 1966.

Most of the basic determinations, measurements, etc., which support the theoretical calculations of the doses received by the S-4 experimental samples have been described in ORNL-TM-1550. The specific gravity of the Neurospora spore suspension used was experimentally determined to be 1.039. The thicknesses of the various layers of absorber which must be considered in calculating the doses to the dry Neurospora spore samples were measured. The spore layer itself had an average thickness of  $11 \text{ mg/cm}^2$ . On each side of this layer was a Millipore filter disc with a thickness of  $4.34 \text{ mg/cm}^2$ . Outside of this on each side was a polyurethane sponge layer with a thickness of  $8.28 \text{ mg/cm}^2$ . Finally, on each side was the chamber window itself, which has a thickness of  $55 \text{ mg/cm}^2$ . Thus there was a total thickness of  $0.073 \text{ g/cm}^2$  to the center of the spore layer.

2.2. Theoretical Dose Calculations. — a. Blood Experiment. Each of the experimental devices for the S-4 blood experiment contained sources with a total activity of 12.5 mCi at 1200 E.S.T. on September 11, 1966. Because the devices could not be assembled simultaneously, the timing of the insertion of the blood samples, and consequently the duration of their "background" exposures was slightly different for the ground control and the flight experiments. The times pertinent to theoretical dose calculation are shown in Table 2.

Because the times involved were not very short in relation to the 14.3 day half-life of  $^{32}\text{P}$ , the isotope decay during the experiment must be taken into consideration. For convenience in calculation, the actual times were converted to "effective" times; i.e., the times required for the same dose if the exposure had occurred at the time the sources were at the nominal full activity of 12.5 mCi per device and there had been no decay during the experiment. This was done by integrating over the time involved:

$$t_{\text{eff}} = \int_{t_1}^{t_2} e^{-\frac{0.693t}{343.2}} dt$$

These effective times are also shown in Table 2. The effective duration of the deliberate radiation exposures were used to calculate the doses (integrated over sample

Table 2  
S-4 experiment timing for calculations of doses received by  
the blood samples in the Gemini XI experiment

	Flight		Ground Control	
	Actual (hr:min)	Effective* (hr)	Actual (hr:min)	Effective* (hr)
Time from nominal activity to insertion of samples in device	14:12	--	16:00	--
Total time samples were in device	80:54	72.5	79:15	71.4
Time from nominal activity to start of irradiation	88:25	--	88:25	--
Duration of irradiation (activation time)	1:10	1.0	1:10	1.0
Total time in device less irradiation time (standby time)	79:44	71.5	78:05	70.4

\*Calculated as described in text.

depth) to the blood samples, using the theoretical considerations presented in ORNL-TM-1550. These theoretical doses are given in Table 3.

Table 3  
Theoretical integrated doses to both flight and ground control  
blood samples in the Gemini XI S-4 experiment

Sample Rank	Dose (rad)
1	68
2	135
3	203
4	270

b. Neurospora Experiment. The source strengths for the experimental devices used in the S-4 Neurospora experiment were the same as those for the blood experiment. Table 4 shows the pertinent times and the "effective times" calculated as for the blood experiment. Theoretical doses to the liquid Neurospora spore suspensions were

Table 4  
S-4 experiment timing for calculations of doses received by the  
Neurospora samples in the Gemini XI experiment

	Flight		Ground Control	
	Actual (hr:min)	Effective* (hr)	Actual (hr:min)	Effective* (hr)
Time from nominal activity to insertion of samples in device	11:26	--	17:00	--
Total time samples were in device	83:29	75.0	78:20	70.0
Time from nominal activity to start of irradiation	51:51	--	51:51	--
Duration of irradiation (activation time)	37:44	32.8	37:44	32.8
Total time in device less irradiation time (standby time)	45:45	42.2	40:36	37.2

\*Calculated as described in text.

calculated in the same manner as for the blood sample exposures, except that allowance was made for the slightly different density of the Neurospora suspension. The calculated doses are given in Table 5.

Calculation of the theoretical doses to the dry spore layers was based on the same considerations as for the liquid samples, except that the different geometry and mass

Table 5  
Theoretical integrated doses to both flight and ground control  
Neurospora samples in the Gemini XI S-4 experiment

Sample Type	Sample Rank	Dose (K rad)
Liquid suspension	1	2.3
	2	4.5
	3	6.8
	4	9.0
Dry spore layer	1	3.4
	2	6.8
	3	10.2
	4	13.6

interposed between the sources and the sample layer were taken into account. One consequence of having a very thin sample layer was that there was very little difference between the maximum, minimum, and integrated doses to the sample volumes. The calculated ratio of maximum to minimum doses for the liquid suspensions is 2.13, and the integrated doses over the entire depth of the suspensions were 64% of the maximum at the inside surface of the chamber windows; for the dry spore layers the calculated ratio of maximum to minimum is virtually unity. The calculated integrated doses to the "irradiated" Neurospora samples are given in Table 5.

2.3. In-flight Dosimeters. — a. Fluoroglass Rods. A pair of silver metaphosphate fluoroglass rod dosimeters (Toshiba) was included within the volume of each of the blood samples and each of the liquid suspensions of the Neurospora spores. These were read out after the flight on a Toshiba Type 3B Fluorometer calibrated against fluoroglass exposed to  $^{60}\text{Co}$   $\gamma$  rays at the NBS. The results are given in Table 6. The figures

Table 6  
 Results of reading the fluoroglass rod and block dosimeters included in each  
 S-4 experiment liquid sample volume and the instrumentation packages

Dosimeter	Sample Rank	Flight Blood	Ground Control Blood	Flight Neurospora	Ground Control Neurospora
Rods*	Control	15.8 ± 2.1	20.0 ± 1.0	12.3 ± 5.5	16.4 ± 1.1
	1	51.5 ± 3.4	48.0 ± 4.5	1,210 ± 28	1,220 ± 28
	2	87.6 ± 5.2	89.8 ± 4.2	2,275 ± 106	2,275 ± 106
	3	136.0 ± 2.2	137.0 ± 5.7	3,340 ± 255	3,050 ± 354
	4	161.0 ± 4.1	155.0 ± 5.3	4,865 ± 375	4,440 ± 368
Blocks†	Battery end	1.2	1.0	1.5	1.4
	Other end	1.4	1.1	1.9	1.7

\*The rod readings are the averages for reading each end of each of the two dosimeters in each sample chamber. The rod readings are in  $^{60}\text{Co}$  R units.

†The block readings are converted to "rad units" as explained in the text.

given are the averages of both ends of both rods in each chamber of each sample holder for the blood device; there was only one liquid sample chamber at each dose for the Neurospora. They are presented in "<sup>60</sup>Co R units", which must be converted to "blood rads" or "Neurospora spore suspension rads" as outlined in section 2.4.a.

b. Fluoroglass Blocks. A pair of 8 x 8 x 4.7 mm fluoroglass block dosimeters (Toshiba) was included in the instrumentation package in each of the S-4 experimental devices as a measure of the Bremsstrahlung within the device. These were also read out following the mission on the fluorometer used for the rod dosimeter readouts. These readings are also shown in Table 6. Most of the dose they registered was Bremsstrahlung from the platinum source plaques which is largely at the 67 eV Pt K fluorescence peak. The fluoroglass is approximately 4.5 X as sensitive in this energy region as it is above 100 eV. The readings of the block dosimeters have been converted to approximate rad units by use of this factor. The block dosimeters from the battery end of each instrumentation package registered a lower dose than those from the other end, as expected because of the different shielding in the two locations. The dosimeters from each of the flight devices showed a higher dose than the ground control devices. This difference, if real, appears to be too large, however, to be explained by the increase in ambient radiation during the mission, since the total ambient radiation dose for the mission has been estimated by NASA to be about 30 mr (on the basis both of a tissue equivalent ionization chamber and of LiF samples carried in the spacecraft).

c. Teflon-LiF Dosimeter Discs. Two 5 mil LiF-impregnated Teflon disc dosimeters (ConRad) were included in each Neurospora spore "sandwich" to measure the doses to the dry spore samples. They were read out on a ConRad TLD instrument after the mission. The instrument was calibrated by means of discs exposed to X-ray doses measured with a Victoreen Condenser R meter which had previously been calibrated at the NBS. The doses indicated by the disc dosimeters are presented in Table 7.

Table 7  
S-4 experiment dry Neurospora spore layer doses indicated by  
LiF-impregnated Teflon disc dosimeters

Experimental Device	Dose Rank	Readout*	Dose Indicated (K rad)	Theoretical <sup>†</sup> (K rad)
Flight	1	764 ± 38	3.4	3.4
	2	1612 ± 80	5.9	6.8
	3	2897 ± 188	9.2	10.2
	4	5017 ± 366	14.1	13.6
Ground control	1	861 ± 8	3.7	3.4
	2	1930 ± 18	6.5	6.8
	3	3520 ± 93	10.1	10.2
	4	4997 ± 1	14.0	13.6

\*Average of two dosimeters for each sample.

<sup>†</sup>From Table 5; "standby" doses negligible, see text.

2.4. Post-flight Dosimetry. — After the S-4 experiment had been carried out, the experimental devices (with the <sup>32</sup>P plaques still in place) were used for various additional dosimetric measurements.

a. Fricke Dosimetry. The same methods were used for absolute measurements by means of Fricke solution of dose rate integrated over the sample volumes as are described in ORNL-TM-1550. Only the highest-intensity positions were measured for each of the experimental devices, since other measurements had already confirmed the 1:2:3:4 source-strength ratios. The average optical density increase per effective hour for the four devices was  $0.00995 \pm 0.00035$ . This value was converted to <sup>137</sup>Cs R equivalents per effective hour by calibration of the Fricke solution against exposure to a <sup>137</sup>Cs  $\gamma$  source measured with a National Bureau of Standards calibrated Victoreen

condenser R meter. Finally, the  $^{137}\text{Cs}$  R equivalents were converted to blood or Neurospora suspension dose rate per effective hour using the considerations outlined in ORNL-TM-1550. The values thus derived are  $277 \pm 10$  rad/hr for the blood samples and  $279 \pm 10$  rad/hr for the Neurospora spore suspension exposed to the highest-activity source plaque array as of 1200 hr E.S.T. on September 11, 1966.

b. Lithium Fluoride Dosimetry. As a further measure of the absolute integrated dose rates to the samples in the S-4 experiments, a series of sample chambers containing loose LiF powder were also exposed in the flight blood experimental device after recovery. Weighed samples of the exposed powder were read out with a ConRad TLD unit. The unit was calibrated with samples of the same lot of LiF given doses of X-rays measured with an NBS calibrated Victoreen condenser R meter. Conversion of the dose rates measured with the LiF powder to blood rad units was based on the relative densities of the two materials. Although some mechanical difficulties were experienced with the TLD readout unit, the results are in satisfactory agreement with the theoretical expectations.

c. Control Sample Dose Rate Determinations. After recovery of the experimental devices, the dose rate per effective hour in the control sample position was measured directly by means of fluoroglass rods (Toshiba) exposed within water-filled sample chambers in the flight blood experimental device. The result was in good agreement with the values obtained in the actual Gemini XI S-4 experiments (Table 6).

The dose to the control samples consisted of both Bremsstrahlung and  $\beta$  particle components. Previous experiments (ORNL-TM-1550) showed that about 67% of the control fluoroglass dosimeter reading is due to the Bremsstrahlung. Allowing for the 4.5 X greater efficiency of the fluoroglass for the Bremsstrahlung, the readings shown may be converted to blood or Neurospora spore suspension doses in rads as shown in Table 8. The same considerations may not, however, be applied directly to the dry Neurospora spore control samples because of their different thickness.

The estimates of control doses given in Table 8 were derived by using the depth-dose information given in ORNL-TM-1550 for relative Bremsstrahlung and

$\beta$  particle doses at the depth equivalent to that of the dry Neurospora layer, and allowing for the 4.5 X greater sensitivity of the fluoroglass to the Bremsstrahlung.

Table 8

Bremsstrahlung and  $\beta$  ray doses received by the control blood and Neurospora spore samples in the Gemini XI S-4 experiments

Sample Type	Device	Bremsstrahlung (rad)	$\beta$ particles (rad)	Total (rad)
Liquid suspension	Flight blood	2.8	5.3	8.1
	Ground control blood	3.0	6.7	9.7
	Flight Neurospora	1.8	4.1	5.9
	Ground Control Neurospora	2.5	5.5	7.9
Dry spore layer	Flight Neurospora	2.1	15.8	17.9
	Ground Control Neurospora	3.4	21.1	24.5

d. Standby Position Dose Estimations. In order to estimate the doses received by each of the blood and Neurospora spore suspension samples while in their "non-irradiating" (standby) positions in the experimental devices during the Gemini XI experiment, a post-flight determination was made with fluoroglass rods (Toshiba). They were placed in a linear array from top to bottom of a series of water-filled sample chambers and exposed in the recovered flight blood experimental device. The resulting readings, together with a rough integration of the volume of sample exposed at various levels were used to calculate the average "standby" exposure for each of the liquid samples in the experiment. The resulting estimates are given in Table 9. No estimates were made for the dry Neurospora spore layers, as their "standby" exposure was certainly negligible in comparison with the large doses received during their deliberate irradiation.

Table 9

Estimates of average doses received by the blood and Neurospora spore suspensions in the Gemini XI S-4 experiments during the total times they spent in the "Non-Irradiating" position in the experimental devices (standby doses) in rad

Device	Dose Rank of Sample			
	1	2	3	4
Flight blood	8.0	10.0	13.0	13.0
Ground control blood	7.9	9.9	13.1	12.8
Flight Neurospora	4.7	5.9	7.9	7.6
Ground control Neurospora	4.2	5.2	7.0	6.7

e. Estimation of Absolute Dose Indicated by Fluoroglass Monitors Exposed During the S-4 Experiment. In order to compare the dose measurements of the fluoroglass rods incorporated in the blood and Neurospora spore suspension sample volumes during the actual Gemini XI experiment with the theoretical expectations based on time and source strength (Tables 3 and 5) it was necessary to determine the contribution of the "standby" exposure to the total reading on the rods. A series of sample holders were filled with water and sealed with dosimeter screws as for the actual Gemini XI experiment. These were given a long exposure in the "non-irradiating" (standby) positions in the recovered ground control blood experimental device. The readouts were converted to blood or Neurospora spore suspension rads per effective hour, and multiplied by the standby time in effective hours for the actual Gemini XI experiments (Tables 2 and 4). These were then subtracted from the average rod readings given in Table 6, yielding the corrected average rod readings presented in Table 10.

The corrected rod readings in  $^{60}\text{Co}$   $\gamma$  rad equivalents were converted to blood or Neurospora spore suspension rad units by dividing by the conversion factor 0.54

Table 10

Comparison of the doses indicated by the fluoroglass dosimeters used as monitors during the actual Gemini XI S-4 experiments with the doses expected from theoretical calculation

Experimental Device	1	2	3	4
	Fluoroglass* theory	Fluoroglass theory	Fluoroglass theory	Fluoroglass theory
Flight blood <sup>†</sup>	77 ± 6	138 ± 11	219 ± 4	268 ± 8
Ground control blood	70 ± 8	143 ± 9	222 ± 11	257 ± 10
Flight Neurospora <sup>‡</sup>	2.3 ± 0.1	4.0 ± 0.2	6.2 ± 0.5	9.0 ± 0.7
Ground control Neurospora	2.3 ± 0.1	4.2 ± 0.2	5.6 ± 0.7	8.2 ± 0.7

\*The fluoroglass rod doses have been corrected for exposure during the time spent in the "Non-Irradiating" (standby) position (see text).

<sup>†</sup> Values are given in rad for blood samples.

<sup>‡</sup> Values are given in K rad for Neurospora spore suspension samples.

derived from experimental considerations as outlined in ORNL-TM-1550. These corrected values, together with the theoretical dose predictions already presented in Tables 3 and 5 are compared in Table 10. The agreement is quite satisfactory, particularly when the possible fluoroglass monitor errors from improper positioning, small bubbles, inhomogeneous  $^{32}\text{P}$  distribution on the plaque, etc. are considered.

### 3. Leukocyte Chromosomes

No evidence of excessive hemolysis or of gross cell damage was seen in the recovered blood samples. The cytological preparations made from the leukocyte cultures were adequate, although there were fewer mitoses in the ground samples than had been hoped for. The cells were scored for chromosomal aberrations in the standard manner used for previous experiments. Altogether a total of 4,340 cells were analyzed. The results are shown in Table 11.

The flight-crew samples show no biologically significant increase in aberration frequencies following the flight. The higher values for chromosome deletions following the flight are not statistically significant ( $P$  between 0.02 and 0.10). The greater frequency of chromatid deletions in the post-flight samples is difficult to interpret, since the cells scored must have been in the pre-DNA-synthesis  $G_1$  stage of the cell cycle at the time of the flight, and the aberrations, if they are in fact induced rather than spontaneous, must have been induced after the cells were in culture. In any case, this result is in agreement with the the results from the Gemini III S-4 experiment, as well as with the results of similar determinations made on leukocyte samples from the flight crews of the other Gemini missions.

The chromosome aberration frequencies seen in the experiment's control blood samples are typical of what is expected in cells exposed to such low radiation doses. They agree well with what was seen in the previous ground experiments, as well as in the control samples from the Gemini III S-4 experiment. There was only the normal incidence of cells with chromosome numbers other than the normal human diploid number of 46. Almost all of the multiple-break aberrations seen were accompanied by the

Table 11  
Results of Gemini XI S-4 experiment chromosome aberration analyses

Subject	Sample	Cells Scored	Est. Dose (rads)	Chromatid Deletions	Chromosome Deletions	Ring and Dicentric Chromosomes
				<u>Crew</u>		
A	Pre-flight	150	---	1	0	0
	Post-flight	200	---	8	7	0
B	Pre-flight	150	---	1	1	0
	Post-flight	200	---	11	5	2
				<u>Experiment</u>		
	Ground	364	10	4	11	1
	Flight	400	8	18	10	8
	Ground	380	76	15	33	17
	Flight	400	76	13	24	20
	Ground	310	145	7	49	43*
	Flight	400	145	21	69	49
	Ground	321	216	5	71	70*
	Flight	400	216	13	84	76
	Ground	265	283	8	94	65
	Flight	400	283	26	106	121 <sup>†</sup>

\*Includes a tricentric scored as two dicentrics.

<sup>†</sup>Includes 1 dicentric ring scored as 1 dicentric and 1 ring.

expected acentric fragments, indicating that the cells scored were virtually all in their first in vitro mitosis, as intended.

A least-squares regression analysis was carried out to obtain the best estimates of the coefficients of aberration production for deletions and for rings and dicentrics in the ground and in-flight portions of the experiment. The chromosome deletion data were fitted to a linear model, while the multiple-break aberration data were fitted to the dose-square model, as was done for the Gemini III data. Of course, the aberration yields from this and most other aberration experiments actually fit more complicated kinetic models better, but the present method of testing the synergism hypothesis gives greater significance to any difference that might be observed than would using the more complicated models, which have more variables. The results of our analyses are shown in Table 12, together with the results of a similar analysis

Table 12  
Coefficients of aberration production for  
Gemini XI S-4 blood experiment

	Deletions ( $\times 10^4$ ) per Cell per rad	Rings and Dicentrics ( $\times 10^6$ ) per Cell per rad <sup>2</sup>
Ground Control	10.22 $\pm$ 0.87	3.84 $\pm$ 0.70
In-Flight	9.01 $\pm$ 0.98	3.64 $\pm$ 0.26
Run 1*	8.16 $\pm$ 1.12	3.22 $\pm$ 0.57

\* Gemini XI mockup experiment number 1 (ORNL-TM-1715, Table 4).

of the data obtained from the first mock-up experiment (Run 1) for comparison. There is no significant difference between the in-flight and ground values either for deletions or for rings and dicentrics. Neither do the values for the Gemini XI S-4 experiment differ from the results of the pre-flight experiment.

#### 4. Neurospora Spores

The data from the analysis of the Neurospora samples irradiated on Millipore filters shows no significant difference in either the survival curves (Fig. 6) or forward-mutation

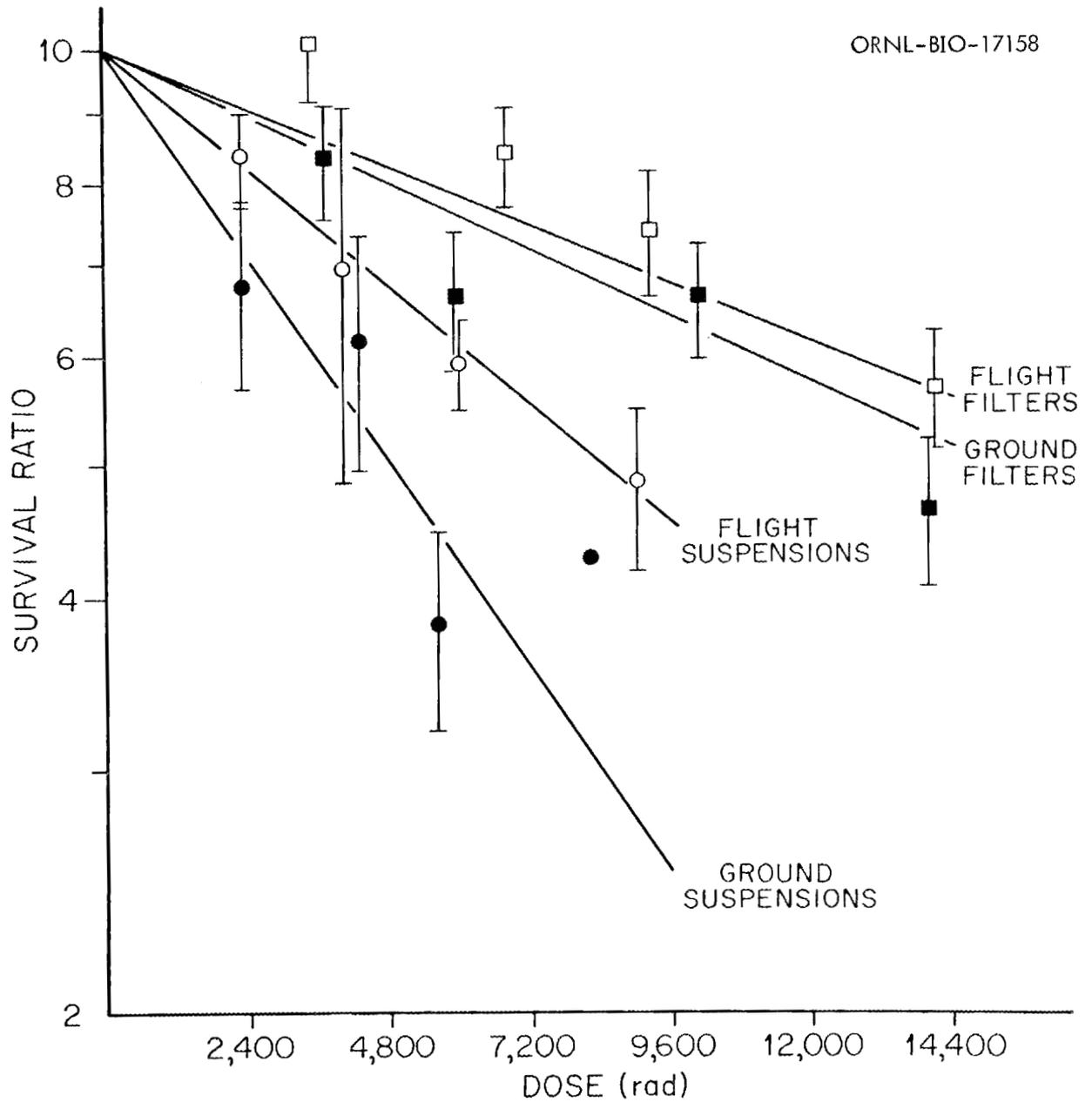


Fig. 6. Survivals of spore samples from the S-4 Neurospora experiment. Circles, suspensions; squares, spore layers on filters. Open points, flight samples; closed points, ground samples.

curves (Fig. 7). The estimates of the forward mutation frequencies obtained in the experiment with  $^{32}\text{P}$   $\beta$  particles are in excellent agreement with chronic 250 kV X-ray

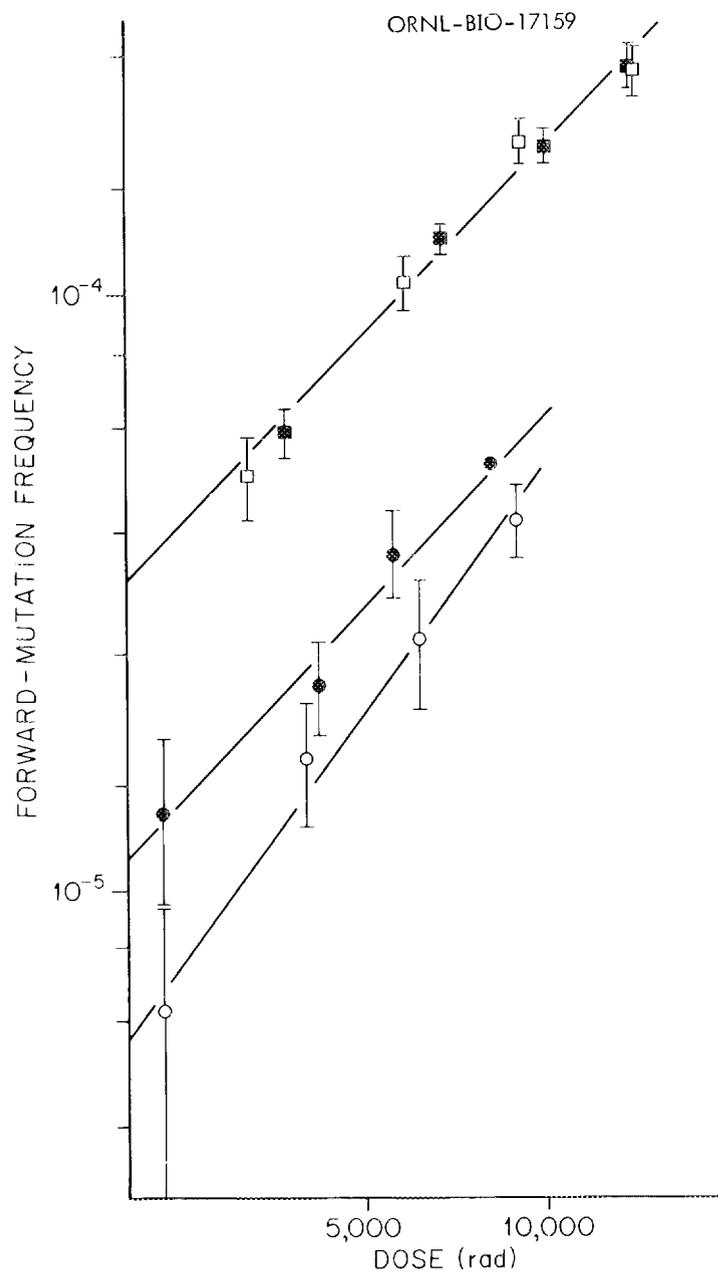


Fig. 7. Forward-mutation frequencies at the ad-3 locus in the S-4 Neurospora experiment. Circles, suspensions; squares, spore layers on filters. Open points, flight samples; closed points, ground samples.

and  $^{137}\text{Cs}$  and  $^{85}\text{Sr}$   $\gamma$  ray exposure data obtained previously. None of the estimates of the slopes of these curves is significantly different from 1.0. The results obtained from the Neurospora samples on Millipore filters indicate that there is not a genetic effect of radiation under conditions of spaceflight that differs from the effect of the same radiation exposure on the ground. In this respect the S-4 Neurospora experiment and the S-4 blood experiment are in good agreement.

Analysis of the data from the Neurospora samples in suspension shows that a significantly higher ( $P = 0.02$ ) survival was obtained in the in-flight samples than in the ground samples. In addition, the forward-mutation frequencies are lower for the in-flight suspensions than for the ground suspensions. This difference, however, is not statistically significant ( $P = 0.09$ ).

### 5. Discussion

The results from the Gemini XI S-4 human blood experiment have failed to confirm the increase in single-break chromosome aberration yields observed in the Gemini III S-4 experiment. The Gemini XI results agree with the Gemini III results both in showing no significant increase in chromosome aberration levels in flight-crew leukocyte samples after an orbital mission, and in failing to demonstrate any increase in multiple-break aberration yields when the cells are irradiated during an orbital flight. While the absolute values of the coefficients of deletion production for the Gemini III and the Gemini XI experiments are different, this difference was to be expected. It is a consequence of the change in the time interval between drawing and irradiating the blood; the experiments carried out prior to the Gemini XI mission consistently showed the same effect.

The S-4 Neurospora experiment was flown with samples collected on filters so that any data could be compared directly with existing information on the genetic effects of chronic and acute exposures to various ionizing radiations. Such samples are actually irradiated under aerobic conditions even if they are in air in sealed containers, because the rate of respiration of the spores on filters is quite low, and the oxygen content of the atmosphere in the sealed container levels off at about 16-17%.

The samples flown as suspensions were included primarily to attempt to mimic the conditions of the S-4 blood experiment as closely as possible. Spores of *Neurospora* in suspension respire rapidly and thus become anaerobic, the actual rate depending on the concentration of the suspension and on the temperature. Fully aerobic suspensions of spores at the concentration used for the S-4 experiment, kept in containers completely impermeable to oxygen, become anaerobic after 75 min if incubated at 25°C.

It is well known that anaerobic conditions protect cells from various effects of ionizing radiations. In *Neurospora*, anoxia resulting from endogeneous metabolism has been shown to give higher levels of survival and lower levels of reverse mutation. Data from previous experiments on aerobic and anaerobic suspensions of the same type as used in the S-4 experiment have indicated that anoxia has the same effect on forward-mutation yields. The data from the S-4 *Neurospora* feasibility experiments clearly show that the anaerobic samples give higher survival levels and lower forward-mutation frequencies than the aerobic samples on filters. Direct measurements of the permeability of the plastic sample holders used for the S-4 experiment have shown that they are somewhat permeable to oxygen. If the spores in suspension were metabolically active during the first part of the Gemini XI mission, the somewhat higher temperature of the spacecraft cabin than the temperature at which the ground experimental device was kept could have resulted both in more rapid diffusion of the oxygen in the experimental device through the sample chamber windows, and in more rapid respiration of the suspended *Neurospora* spores. It seems possible, then, that the differences between the in-flight and ground suspension samples may have resulted from the spores having been in a somewhat different state of anoxia through at least part of the irradiation period.

The simplest interpretation of the results of the Gemini III and Gemini XI S-4 experiments is that the significant increase in chromosome deletion yields seen in the Gemini III experiment was the result of a statistical sampling accident (which, while unlikely, certainly had a finite probability of occurring). If it is argued that the difference in the Gemini III deletion results actually reflects a real synergistic effect,

then the conditions under which such an effect can occur must be very special; the Gemini III and XI mission profiles certainly contain the same major elements of vibration, "weightlessness", etc. A third possibility, that the lack of a significant difference in the Gemini XI blood results is itself due to a statistical sampling error, seems especially unlikely in view of the results of the S-4 Neurospora experiment.

The S-4 experiment has shown, in contrast to the reports which led to the synergism hypothesis in the first place, that neither orbital spaceflight nor any of the stresses connected with it produce significant unpredicted genetic damage, at least in so far as chromosomal aberration production, cell killing, and forward-mutation are valid measures of this general type of effect. Furthermore, the Gemini XI results lead us to conclude that no synergistic effect exists between radiation and other factors associated with spaceflight.



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