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SYNERGISTIC EFFECT OF ZERO-G
AND RADIATION ON WHITE BLOOD CELLS
AN EXPERIMENT FOR THE
GEMINI III MANNED SPACE FLIGHT
ANNUAL REPORT
PERIOD ENDING JUNE 30, 1964

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BIOLOGY DIVISION

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

**An Experiment for the
Gemini III Manned Space Flight**

Annual Report
Period Ending June 30, 1964

Prepared by
M. A. Bender

This research is carried out at ORNL under NASA Order Number R-104 Task 4

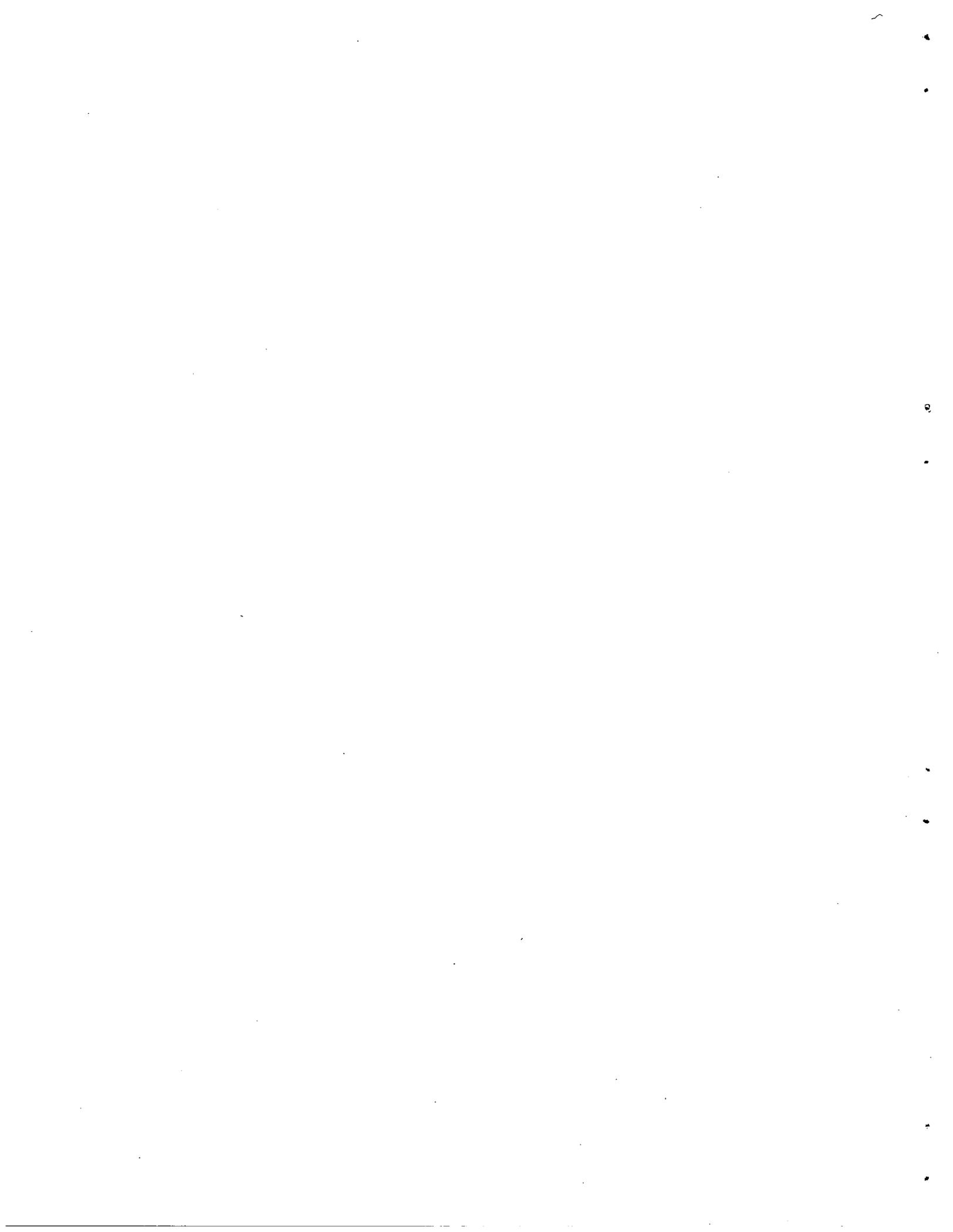
JANUARY 1965

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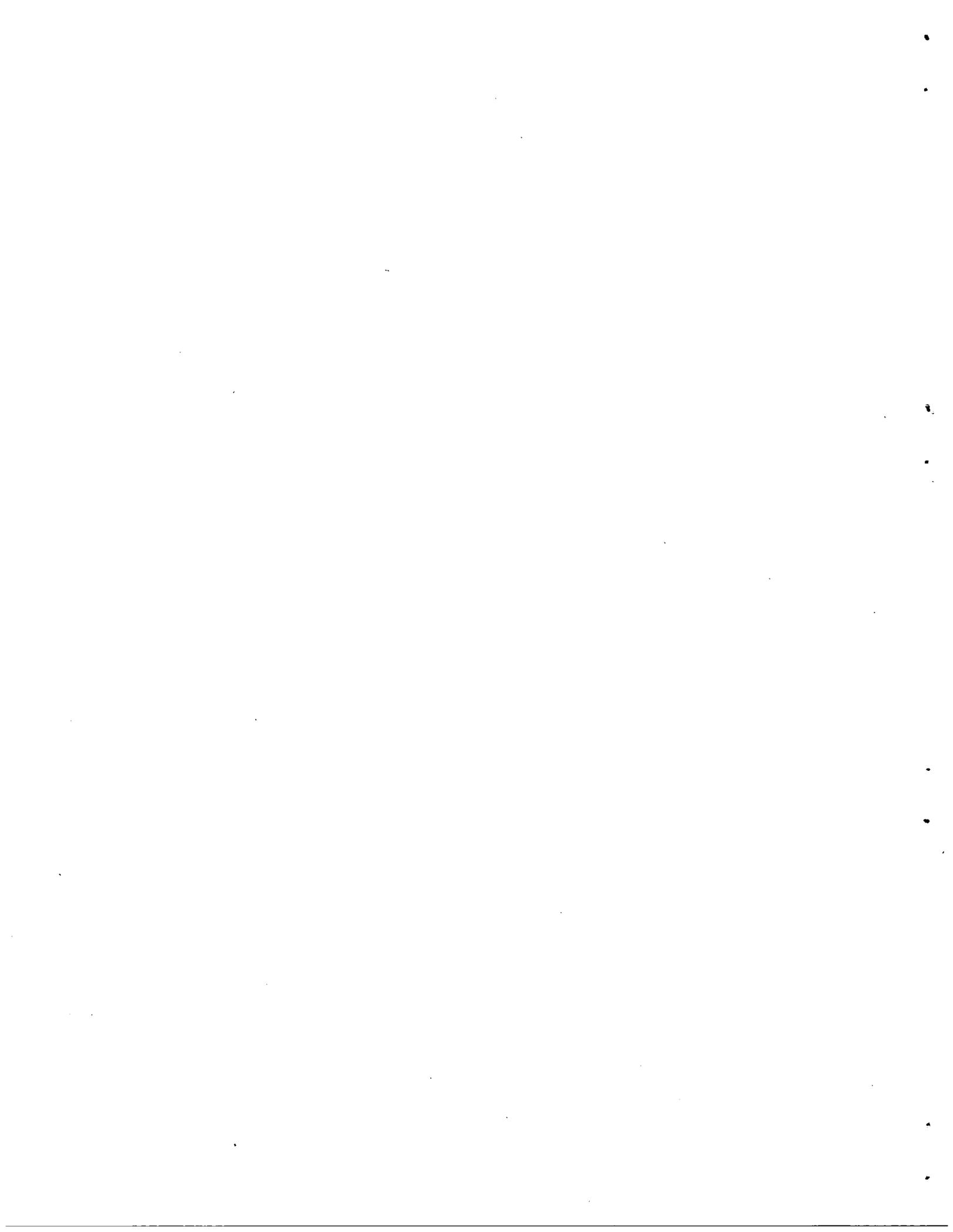


SUMMARY

This report describes work performed through June 30, 1964, on an experiment entitled "Synergistic Effect of Zero-G and Radiation on White Blood Cells." The experiment is being 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. The work consists of the design and execution of a single experiment which will be carried on the Gemini III manned space flight. The experiment consists of the irradiation of samples of human leukocytes with ^{32}P beta particles during the orbital phase of the space flight and the subsequent cytogenetic analysis of the material to determine chromosomal aberration rates. Preparation of the experiment includes design, fabrication, and testing of the necessary hardware and procurement of the required

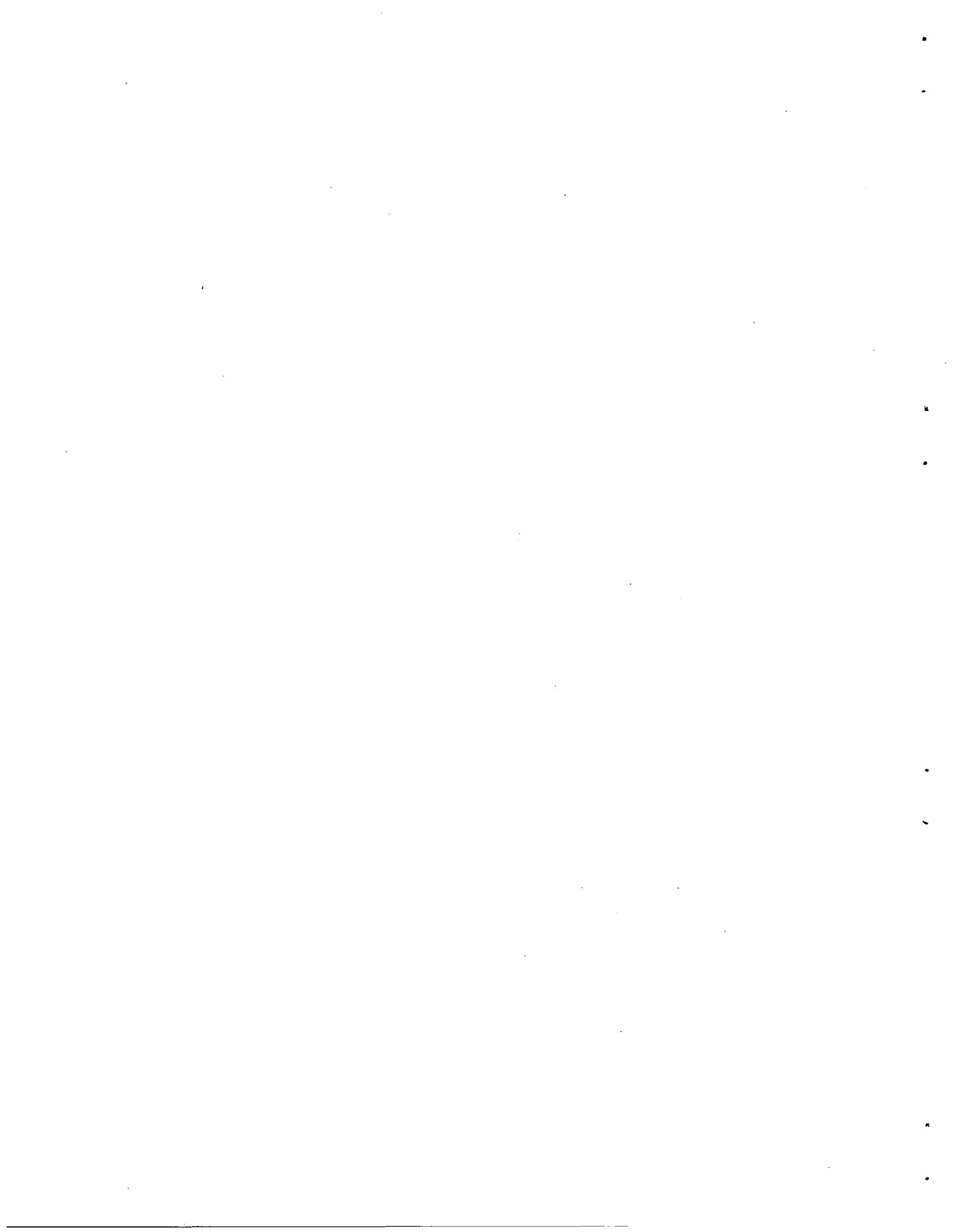
auxiliary equipment.

The project was begun during January 1964. The Gemini III mission is now scheduled for December 1964. It is anticipated that the project will be completed during the first quarter of calendar year 1965. During the last half of FY 1964, both the experimental design and the experimental device have been brought to their final form. Experimental devices, together with the required documentation, have been delivered to the NASA. Biological, radiological, and physical testing have been largely completed. Much of the equipment necessary for the preflight preparation and the postflight handling of the experiment has been procured or fabricated. Additional experimental devices are being fabricated, and the personnel required for the field operations are now being trained.



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I. Introduction

The Biology Division of the Oak Ridge National Laboratory has, for several years, actively participated in several types of space-oriented radiobiological research.¹ In brief, Biology Division experiments were flown on the "BIOS" ballistic deep-space probes in 1961, and on a series of high-altitude balloon flights in 1962. Also, the Biology Division has been conducting measurements of the relative biological effectiveness of protons of a wide range of energies since 1962. Much of this research has been performed under contracts between the NASA and the USAEC. In addition, the Biology Division has proposed a number of radiobiological experiments for inclusion on future space flights. Several of these have been accepted for the proposed "Biosatellite" series of flights. One of the proposals, intended for these flights, was to measure chromosomal aberration frequencies in human white blood cells irradiated with gamma rays while in orbital flight.

¹Oak Ridge National Laboratory Technical Manual ORNL-TM-720.

On December 27, 1963, NASA personnel contacted the Biology Division and requested a new informal proposal for a human leukocyte experiment modified to make it suitable for inclusion on the Gemini III manned space flight, scheduled for late 1964. The necessary information was supplied to the NASA on December 30, 1963. On January 11, 1964, the Biology Division was again contacted by the NASA and was requested to supply a "hard mockup" of the experiment to the Houston Manned Spaceflight Center as soon as possible. The Biology Division then arranged with the Oak Ridge Y-12 Plant to do the necessary design, testing, and fabrication work, which was begun on January 15, 1964. Experimental devices with two different geometries (designated BIG-I and BIG-II) were delivered on January 27, 1964. Although both models seemed satisfactory, BIG-I was selected for the flight, primarily because testing had progressed further. In early February 1964, the USAEC requested financial support for the project from the NASA. This support was granted in April, and full-scale work on the project was then started.

II. Organization

The development and execution of the "Synergistic Effect of Zero-G and Radiation on White Blood Cells" experiment is quite complex and has had to be performed on a very limited time schedule. It has therefore required the close cooperation of several different Oak Ridge organizations. One of the major elements in the program is the design, fabrication, and testing of the experimental device itself (BIG-I) and of the necessary supporting equipment. This work is the responsibility of the Oak Ridge Y-12 Plant, which possesses unique capabilities and facilities for this type of work. Development and fabrication of the required isotopic beta radiation sources are being handled by the Isotopes Division of the Oak Ridge National Laboratory. The conceptual design of the experiment, the execution of the biological experiment

(including the necessary biological testing), and the radiological testing are being done by the Oak Ridge National Laboratory Biology Division. The individuals directly responsible for the various phases of the project are as follows:

Principal Investigator: M. A. Bender, Biology Division, ORNL

Physical Design, Fabrication, and Testing:

H. F. Smith, Jr., Oak Ridge Y-12 Plant

Mechanical: W. T. Smith, Jr.

Testing: W. W. Lee

Instrumentation: S. E. Groothuis

Isotopes Sources: F. N. Case, Isotopes Division, ORNL

Biology: P. C. Gooch, Biology Division, ORNL

Radiological Physics: S. Kondo, Biology Division, ORNL

III. Experimental Design

A. THE BIOLOGICAL PROBLEM

Biological effects, of types usually associated with radiation damage, have been observed after both ballistic and orbital space flights.² These effects have included mutation, chromosomal aberration production, and cell killing. In many cases the levels of effect noted were many times greater than would have been predicted from the radiation exposures received during flight. Unfortunately, many of the observations are difficult to interpret, either because they are not completely reported, or because the design of the experiment makes the results ambiguous. Nevertheless, the question has been raised of whether there is a radiation or radiation-like hazard associated with space flight. This possibility has received considerable attention both by the NASA, to whom it is a matter of practical concern, and by radiobiologists interested in the possible mechanisms. Such an unpredicted radiation effect, if one actually exists, might be due to either or both of two things. Since one component of the radiation encountered above the earth's atmosphere (the "heavy primaries") is not available for study in terrestrial laboratories, the possibility exists that these particles have unexpected biological effects. The other possibility is that other environmental features of space flight, such as prolonged weightlessness, interact synergistically with radiation to produce unexpected effects.

The only test of these possibilities is to perform a radiobiological experiment during a space flight. Merely analyzing material flown on a space mission and comparing it with ground control material is not satisfactory, because the effects noted, if any, cannot clearly be assigned to radiation damage. A practical experiment is to irradiate a thoroughly studied biological material with a known quantity and quality of radiation during

the "zero-g" phase of an orbital space flight. The types and frequencies of effects may then be compared with those observed in suitable "in-flight" and ground-based controls. An increased effect in the experimental material, *including* the "flight control," would be evidence for an effect of either the ambient radiation or some other space flight parameter. An increased effect in the experimental material, but *not including* the "flight control," on the other hand, would be evidence for a synergism between radiation and some other space flight parameter. Analysis of the types and kinetics of responses in a thoroughly studied material would probably lead to an understanding of the mechanism of any such unpredicted effect. This experimental plan has, in fact, recently received the approval of a Panel on Radiation Biology created by the National Academy of Sciences-National Research Council Space Science Board.³

The experimental material selected for the present experiment is freshly drawn whole human blood. The parameters to be measured are the frequencies of the various types of single- and multiple-break chromosome aberrations. This experimental system is very well suited to the purpose. A great deal of experience gives us a thorough knowledge of the behavior of chromosome aberrations in relation to dose, dose rate, quality of radiation, and various environmental factors in a wide variety of experimental materials. Human peripheral leukocytes can be made to undergo mitosis in short-term tissue cultures stimulated by the substance "phytohaemagglutinin," thus making it possible to analyze their chromosomes. The cells which divide in these cultures are all in the uniformly radiosensitive pre-DNA-synthesis stage of the cell cycle before they are put in culture. The chromosomal aberrations induced by irradiation of whole blood are thus of the easily scored chromosome type and

²See, for example, *Problemy kosmicheskoy biologii*, Vol. I, ed. N. M. Sisakyan, U.S.S.R. Academy of Sciences Publishing House, Moscow, 1962 (NASA TT F-174) and Vol. II, ed. N. M. Sisakyan and V. I. Yazdovskiy, U.S.S.R. Academy of Sciences Publishing House, Moscow, 1962 (JPRS: 18,395; OTS: 63-21437).

³Position Paper on Theoretical Aspects of Radiobiology as Applied to the Space Program, Environmental Biology Committee Panel on Radiation Biology, Space Science Board, NAS-NRC, Washington, 1963.

are clearly distinguishable from the spontaneous chromatid-type aberrations arising during culture. The numerous experimental studies of the Biology Division provide a good background of knowledge of the particular responses of the human leukocyte system to various types of radiation, as well as practical experience in handling the system under difficult field conditions.⁴ In addition, the use of human material offers the practical advantage of direct application to the question of space-flight hazards.

B. EXPERIMENTAL DESIGN REQUIREMENTS

The experimental material selected, whole heparinized human blood, dictates certain features of the experimental design. It has been found that the minimum blood volume with which it is practical to work is about 3 ml. The cells in the blood must be protected from freezing, and their temperature cannot be allowed to exceed approximately 40°C. Because the cells consume oxygen, a small bubble of "reserve" air must be included in the sample container. Excessive evaporation of the sample must be prevented. The blood sample must be kept sterile, and the sterilized blood-sample container must not be toxic to the cells. Experience indicates that to be sure of successful culture of the leukocytes after the experiment is recovered, no more than about 24 hr can be allowed to elapse between the time the blood is drawn and the time the cells are put into culture.

The effect to be measured also influences the experimental design. Single-break chromosome aberrations increase as a linear function of the radiation dose. Unless the linear energy transfer of the radiation employed is quite high, chromosome aberrations involving two breaks increase approximately as the square of the dose, and aberrations involving greater numbers of breaks as

correspondingly higher powers of the dose. Ignoring those aberrations involving more than two breaks, which are in any case infrequent at low and moderate doses, the yield of aberrations may be approximated by the expression

$$Y = a + bD + cD^2,$$

where Y is the yield, a the spontaneous aberration frequency, D the dose, and b and c the coefficients of single- and two-break aberrations respectively. In order to fit the experimental data to such a three-parameter model, at least four points on a dose curve are required. Both as protection against loss of one of the samples, and because the "in-flight" control is actually a measure of all effects other than that of the deliberate radiation exposure, a dose curve including four samples deliberately exposed to four different doses, plus the "in-flight" control, was selected. Nominal doses of 50, 100, 150, and 200 rad were selected on the basis of previous experience with the human leukocyte system. The expense and importance of the experiment early led to the adoption of a redundancy requirement. Therefore it was decided to fly two complete sets of samples, with blood from a different donor for each set, thus ensuring that the failure of an individual's blood cells to grow in culture, or the loss of single samples from both, would not prevent successful completion of the experiment. The experimental material thus consists of ten 3-ml blood samples having an aggregate volume of 30 cc and a total mass of approximately 30 g. The "ground control" consists, of course, of a complete duplicate set of ten samples.

The Gemini III space flight, for which the experiment is scheduled, is the first of the manned Gemini missions. Thus not only sensitive spacecraft components and instruments, but the astronauts themselves must be protected from the radiation source used to irradiate the blood samples. Since the NASA informed us that the complete experimental package must be as small as possible and have the smallest mass possible, the weight of the required shielding dictated the use of beta rays for the experiment. Phosphorus-32 was selected as the source of beta rays because it emits only a single beta particle, because the particle's energy (average $e = 0.7$ Mev) is suitable, because ³²P has already been used extensively in radiobiological work, and because the isotope is

⁴Bender, M. A. and P. C. Gooch, 1962, *Proc. Natl. Acad. Sci. (Wash.)* **48**, 522-532; Bender, M. A. and D. M. Prescott, 1962, *Exptl. Cell. Research* **27**, 221-229; Bender, M. A. and P. C. Gooch, 1962, *Cytogenetics* **1**, 65-74; Bender, M. A. and P. C. Gooch, 1963, *Cytogenetics* **2**, 107-116; Gooch, P. C., M. A. Bender, and M. L. Randolph, 1964, *Biological Effects of Neutron and Proton Irradiations*, Vol. I, IAEA, Vienna, pp. 325-342.

easily produced in usable form. Because the exact nature of the effect being investigated is unknown, it was necessary to place more or less arbitrary restrictions on those experimental conditions not already dictated by the flight profile for the Gemini III mission. It was decided that the radiation exposure would be both preceded and followed by at least 1 hr of "weightlessness." The rate at which the dose is to be given will be between 1 and 10 rad/min (it is of necessity different for the different doses). The maximum inhomogeneity of dose over the sample volume will be $\pm 50\%$. Because the induction of chromosome aberrations is known to be dependent on oxygen tension, it was decided that the samples would be irradiated in air, rather than in the oxygen environment of the spacecraft. It was also decided that, although no attempt would be made to control the temperature at the time of irradiation, other than to keep it within the limits required to keep the cells alive, a record of the temperature throughout the flight would be made by instruments included in the experimental device. The instrumentation will include both dosimeters exposed with each blood sample and a recording of the times at which the irradiation was started and stopped.

The geometrical arrangement adopted to meet the requirement for relatively homogeneous irradiation over the sample volume is to place the blood samples in roughly tissue equivalent chambers in the form of a disk 3 mm thick, and to irradiate this disk simultaneously from both sides. Thus as the dose from the source on one side of the sample is decreasing with distance from that source, that from the source on the other side increases, and a relatively homogeneous flux is achieved over the entire sample volume.

C. EXPERIMENTAL PLAN

Blood will be drawn from two donors, one male and one female, about 6 hr before the scheduled liftoff time of the Gemini III vehicle. Sterile heparinized 3-ml samples will be loaded into sterile chambers which will then be assembled into two identical BIG-I devices, each containing a total of ten such samples. One BIG-I device will be placed in the Gemini III vehicle 2 hr before liftoff; the other will remain at the assembly site

near the launch complex. In the event of excessive holds during the launch countdown, a second pair of BIG-I devices will be loaded and substituted for the original set. In this way the blood samples will be relatively "fresh" even if the launch is significantly delayed, thus allowing the maximum time for possible delays at the recovery end.

During the second of the scheduled three 90-min orbits of the Gemini III mission, one of the astronauts will begin the irradiation of the blood samples by moving a handle on the BIG-I device. He will report his action, if possible in real-time, and the "ground control" device will be similarly actuated. At the proper time, approximately 30 min later, and still within the second orbit, the astronaut will stop the irradiation by again moving the handle of the BIG-I device. The exact time required for the irradiation will be determined by the source activity at the time of liftoff, and will be calculated after the time of liftoff is known. The astronaut will report, again in real-time if possible, that he is stopping the irradiation, and the "ground control" device will also be deactivated at the proper time.

After recovery of the Gemini III vehicle, the BIG-I device will be opened and the blood samples recovered and put in culture aboard the recovery vessel. Simultaneously, the "ground control" samples will also be placed in culture at the launch site. Seventy-two hours after the cultures are made, the cells will be fixed and cytological preparations made. These preparations, together with the dosimeters and other instruments, will be returned to Oak Ridge for analysis. From each of the 20 blood samples, 150 metaphase figures will be analyzed for chromosomal aberrations. Data from a total of 3000 cells (138,000 chromosomes) will thus be available for statistical analysis.

Both the "flight" and the "ground control" data will be fitted to the model

$$Y = a + bD + cD^2$$

by means of least-squares nonlinear regression analysis, using an iterative digital computer technique. If indicated, a "site number" analysis of the data will also be made. If no statistically significant differences between the "flight" and the "ground" results are demonstrated, the maximum value of synergism between the space-flight parameters and the deliberate irradiation will be determined

at the 95% confidence level. If there are statistically significant differences between the "flight" and the "ground" results, the degree of synergism will be determined.

A supporting study, not directly connected with the BIG-I experiment, is also planned. Blood samples will be obtained from the astronauts chosen for the Gemini III mission shortly before the flight. Postflight samples will also be obtained

aboard the recovery vessel shortly after the mission is completed. These will be handled in the same way as the BIG-I experimental blood samples, and aberration analyses will be made of the chromosomes of these pre- and postflight samples. The data from the two sets of samples will be compared in order to determine whether a detectable increase in the chromosomal aberration frequency was caused by the flight.

IV. Experimental Device

Two different experimental devices were designed and constructed for submission to the NASA Gemini Project Office (GPO). These were designated BIG-I and BIG-II. They differed only in geometry—BIG-I operating through a linear, and BIG-II through a rotary, motion of the blood samples. Development of both was pursued because of uncertainty about possible restrictions on the geometry of the space available for the device aboard the Gemini III vehicle. Figures 1 and 2 are photographs of the BIG-I and BIG-II devices delivered to the GPO in January 1964. Figures 3 and 4 show their internal design. Of the two models, BIG-II is slightly more economical of space; it contains no "dead" volume, such as is

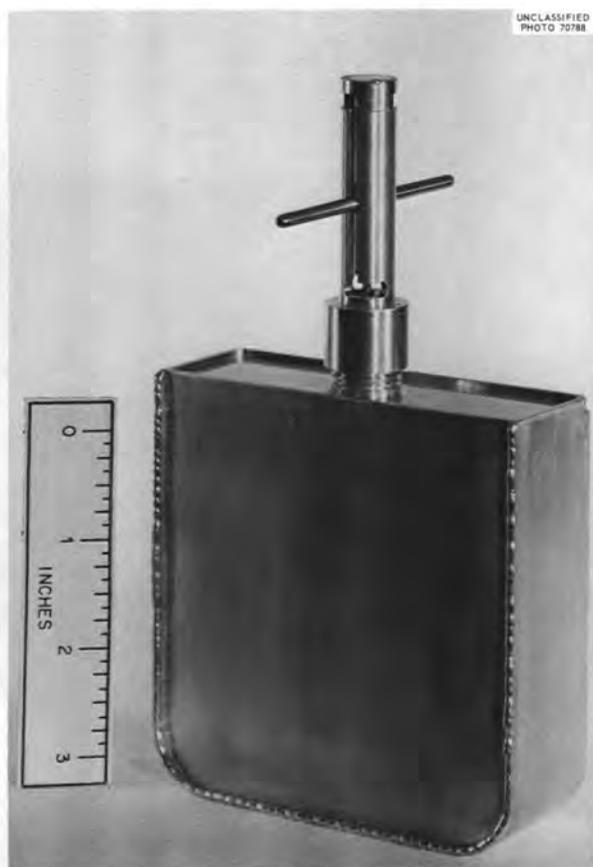


Fig. 1. Photograph of the First Model of the BIG-I Device. Note the unwelded top joint.



Fig. 2. Photograph of the BIG-II Device. Note unwelded upset joint around bottom perimeter. The activating lever is unlocked by pushing down and pulling out; a 90° rotation of the lever starts the irradiation.

necessary in the BIG-I device in order to allow for the linear motion of the blood samples. An additional advantage of the BIG-II design is its hermetic seal, made possible because the operating motion is transmitted to the interior through a "wobble bellows." BIG-I was selected for the flight, however, because its testing had progressed further at the time of delivery. As delivered, loaded with blood specimens, BIG-I weighed 321 g and BIG-II 328 g, without the instrument package. BIG-I had been tested and met the requirements for qualification for the flight. Either model could have been flown, as delivered, with the addition of an approximately 30-g instrument package.

A. THE BIG-I DEVICE

Several modifications of the BIG-I device have been made since the first model was delivered to the GPO. Since the mass of the device is apparently not as critical as had originally been believed, the GPO requested that the operating handle assembly be made somewhat heavier in order to allow easier

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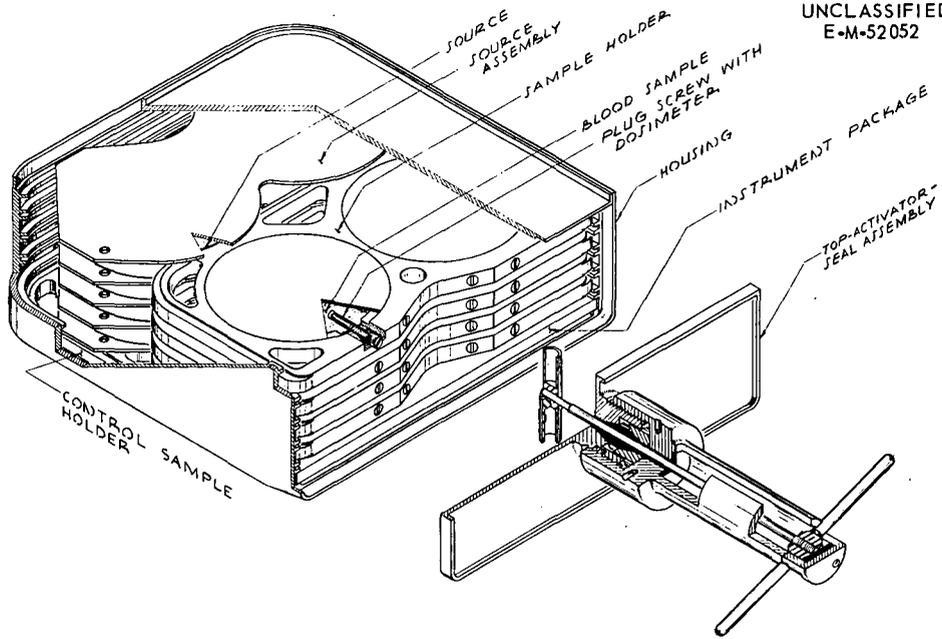


Fig. 3. Cutaway Drawing Showing Design Features of the First Model of the BIG-I Device.

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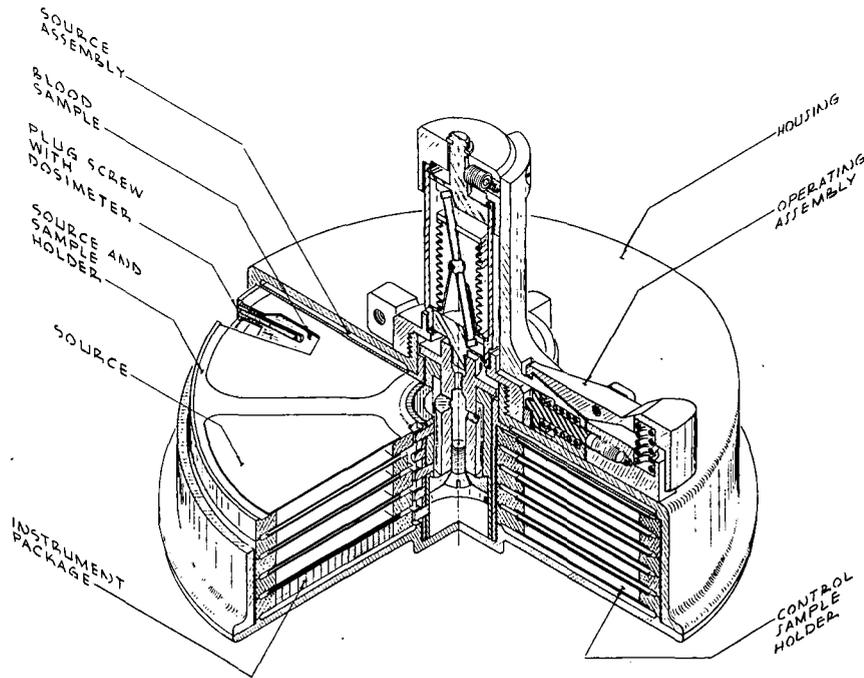


Fig. 4. Cutaway Drawing Showing Internal Construction of the BIG-II Device. Note the complete absence of "unused" space, and also the "wobble bellows" which allows rotary motion to be transmitted through hermetic seal.

manipulation by an astronaut with gloved hands. Also, further experimentation has led to the adoption of a more economical blood-sample holder of simpler construction than the original model, which had Mylar-covered aluminum foil "windows." Figure 5 shows the BIG-I device in its final form, with one side removed in order to show the internal details. The individual parts and subassemblies are shown in Fig. 6. These items include the top and operating mechanism (item 1), the housing (items 2 and 3), the blood-sample holders (item 4), the ^{32}P source plates (item 5), and the instrument package (item 6). Figure 7 shows these parts in a cutaway view; Figs. 5 and 7 both show the geometry of the alternating array of source plates and blood-sample holders within the device. The position of the blood-sample holders shown is the "nonirradiating" position. During irradiation, the blood-sample holders are aligned between the sources.

The irradiation geometry of the BIG-I device is a relatively simple one. Figure 8 is a cross section through the device, showing the essential features of this arrangement. There are five source-plate assemblies located in the "bottom" of the housing. Four blood-sample holders, each with a pair of blood samples, slide in grooves in the housing side walls, and can be slid until they lie between the source plates. The source plates are coated with a thin layer of ^{32}P . Their activities are matched for pairs "seeing" the same blood-sample holder, and the activities of the pairs of sources are arranged in the ratio of 1:2:3:4 in order to give the four doses in the same time. A total of less than 20 mc of ^{32}P is required. The mass of a source plate is high enough to stop most of the beta particles from the source on one side from traveling through the plate to the other side, thus keeping interaction between adjacent blood-sample-holder irradiation fields at a minimum. While in the "nonirradiating" position, there is enough material in the edge of the blood-sample holder to prevent most of the beta particles leaving the edge of a source plate at an angle from entering the blood sample. The control blood sample occupies a fixed position on the "cold" side of one of the end source plates and is shielded from the beta particles from the "hot" side by the mass of the source plate itself. In the "irradiating" position, each of the experimental disk-form blood samples is located in the beta particle field between a pair

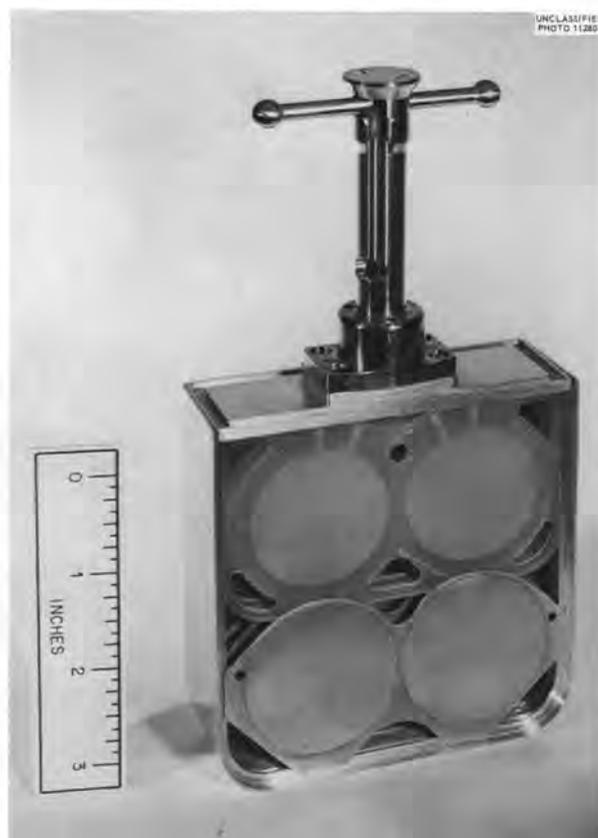


Fig. 5. Photograph of the BIG-I Device as Modified to Make Operating-Handle Manipulation Easier. Side has been left off to show the source plates and the blood sample chambers (in the nonirradiating position).

of parallel-plane sources which extend far enough beyond its edges to eliminate most of the "edge effect."

The exact design and construction details of each part of the BIG-I device represent a compromise between the requirements of the experiment, the structural features required to obtain sufficient strength and dependability with the minimum mass and volume, and the availability and expense of materials and fabrication techniques. The major component items will be considered individually.

Housing (Fig. 9).—The body of the housing of the BIG-I device is machined from a single piece of type 6061 aluminum. A side plate and top of the same material complete the assembly. The housing forms the framework for the device; the source-plate slots and slideways for the blood-sample holders are milled in as integral parts. Together with the top and side plate, the body of the housing forms an enclosure which serves

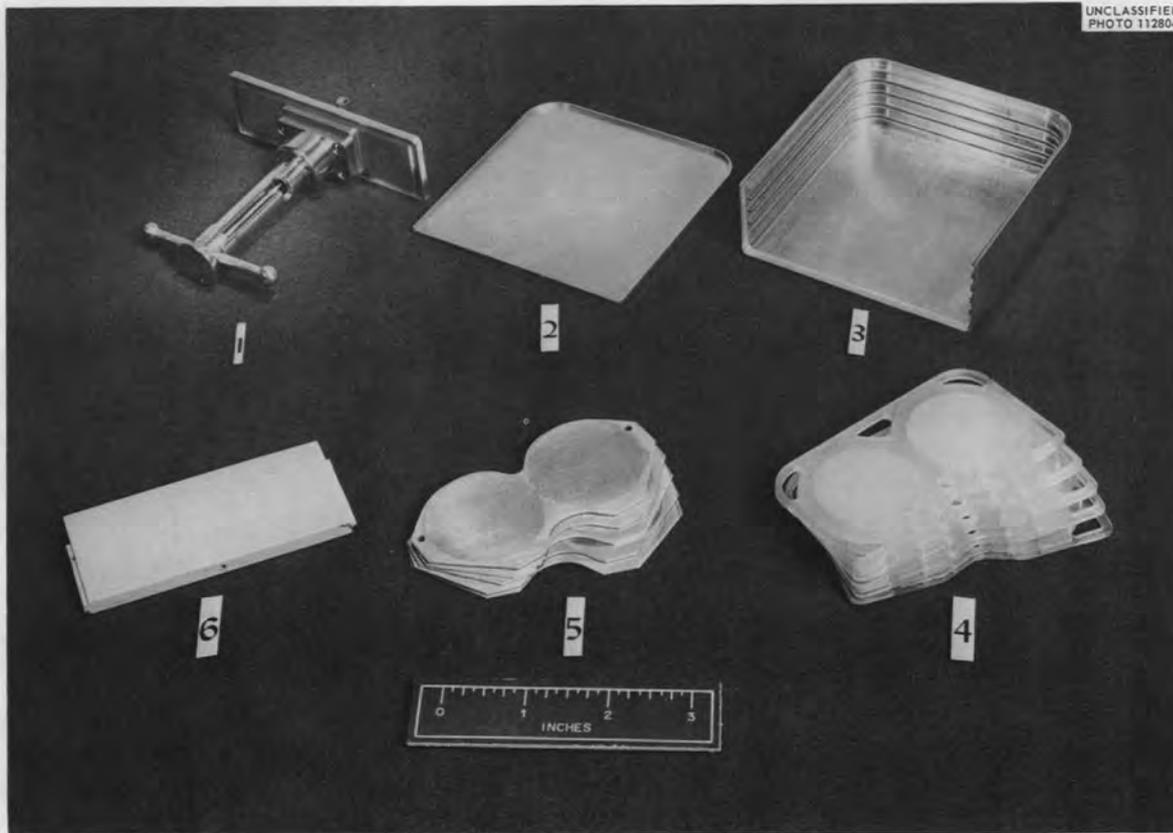


Fig. 6. Component Parts of the BIG-I Device.

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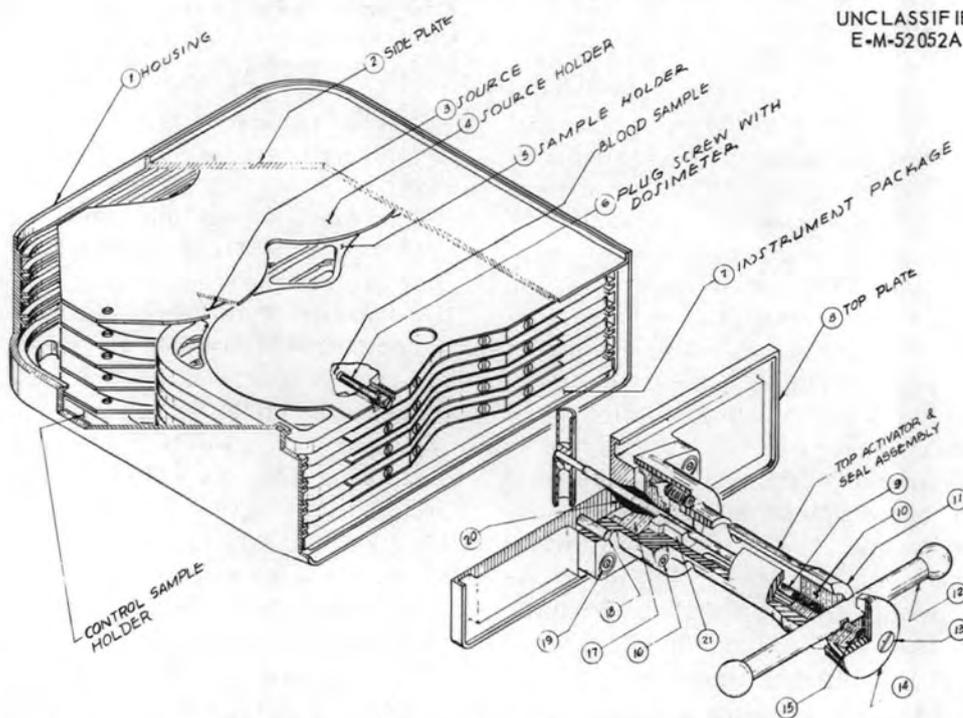


Fig. 7. Cutaway Drawing of the Modified BIG-I Device Showing Details of the Modified Operating-Handle Assembly.

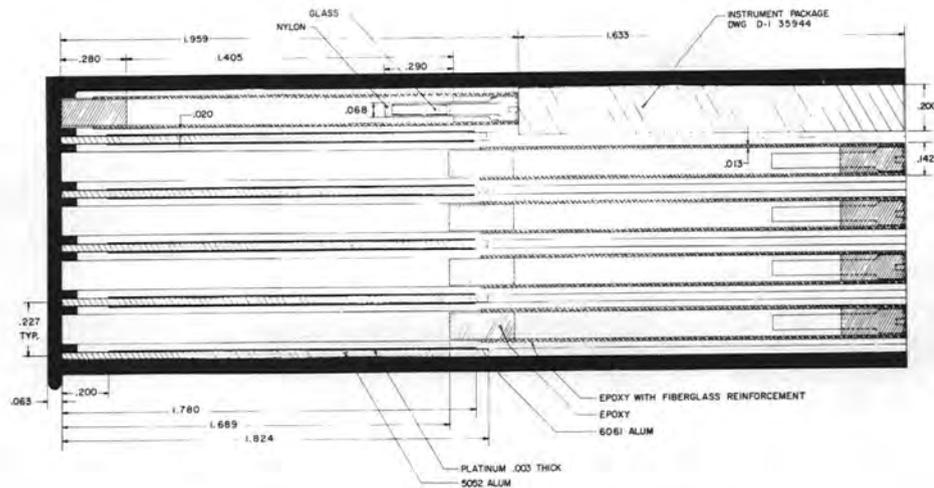


Fig. 8. Cross-Sectional Plan of the BIG-I Device Showing the Geometrical Arrangement of Components.



Fig. 9. BIG-I Housing Parts.

both as a shield for the beta radiation from the ^{32}P sources and as an environmental chamber for the experimental material; it also constitutes sufficient containment to prevent the escape of the radioisotope into the spacecraft cabin in the event of accidents occurring during the mission. Aluminum was selected for the housing both for its low Z -number, which helps to minimize the induction of soft bremsstrahlung x rays by incident beta particles, and also to minimize the mass of the device. The side plate is assembled to the body of the housing by Heliarc welding of an upset-type joint. The assembly is tested for vacuum-tightness

after welding. After loading the source plates, blood-sample holders, and instrument package, the top is also Heliarc welded in place. This operation, together with leak testing of the weld, is performed in the field at the launch site just prior to insertion of the device into the space craft. After the experiment is completed, the BIG-I devices are opened by cutting through the housing top weld with a motor-driven tool.

^{32}P Source-Plate Assemblies (Fig. 10).—The source-plate supports are fabricated of type 5052 aluminum. They both physically support the source plates and provide some of the mass required to stop the beta particles from passing through to the “back” side of the assembly. The source-plate supports are supported at four points by tabs which fit into slots in the housing. They are held in place in the slots by Eastman 910 adhesive. In each plate are two small holes which engage studs on a special loading tool that facilitates assembly into the housing. The source plates themselves are 4-cm disks of 0.003-in. platinum. Platinum was chosen, in spite of its very high Z -number, because it is an excellent beta-particle reflector. Thus the increase in bremsstrahlung radiation due to the high Z -number is offset by the fact that the increase in the beta flux due to reflection allows the use of less ^{32}P , with consequent decrease in bremsstrahlung. The platinum disks are assembled to the support plates by means of

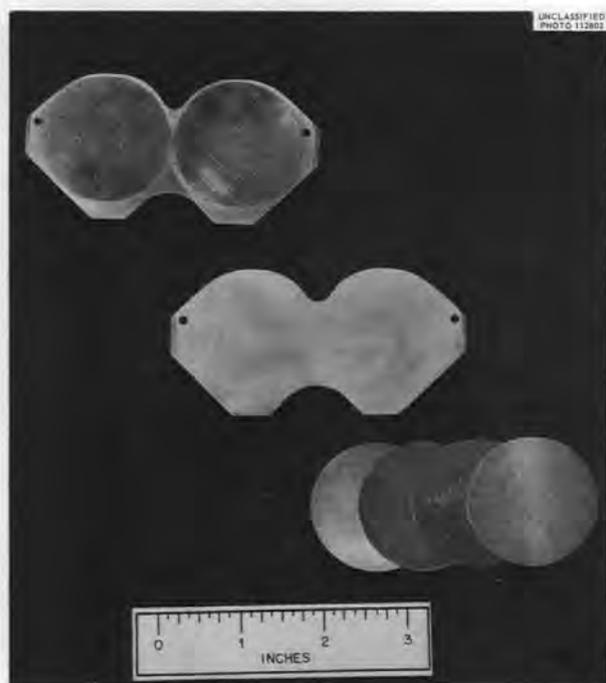


Fig. 10. BIG-I Source-Plate Assembly and Component Parts.

Pliobond adhesive, which tests showed would withstand the radiation fields to which it is exposed. The ^{32}P is applied in a solution of potassium silicate; the activity per disk is controlled by volumetric measurement of dilutions of a solution of known activity. The aliquot of liquid is spread evenly over the surface of the disk and, after drying, forms a hard silicate layer in which the required amount of ^{32}P is evenly distributed. Three of the source-plate assemblies have ^{32}P sources on both sides; the end ones have sources on one side only. The end source-plate assembly nearest the control blood-sample holder has non-active disks on the "back" side to help shield the samples from the ^{32}P sources.

Blood-Sample Holders (Fig. 11).—The blood-sample holders presented some design problems because they must be sterilized and they must be nontoxic, because the "windows," while being sufficiently strong to withstand considerable pressure in the event of a loss of housing pressure, must have a low mass per unit area, and because they must be constructed of a material of a low average Z -number in order to avoid excessive bremsstrahlung radiation. After thorough biological testing, EPON 828 epoxy resin cured with TETA catalyst

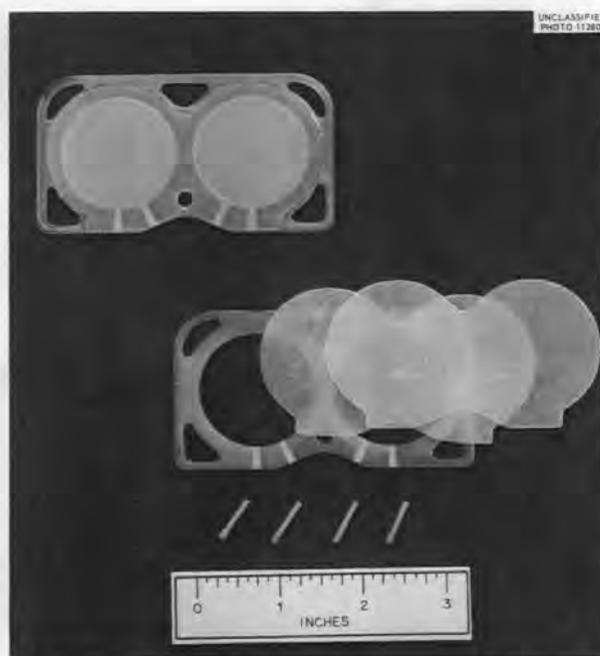


Fig. 11. BIG-I Blood-Sample Holder and Component Parts.

was finally selected. The body of the holder is cast and machined to final dimensions. The "windows" are molded of the same plastic with a Fiberglas reinforcement and "glued" to the body by means of the same epoxy material. Each assembly is leak tested to 4 psig, and then cleaned, packaged, and sterilized with ethylene oxide gas. The four chamber assemblies for the blood samples to be irradiated are attached to the operating handle of the BIG-I device by means of a "draw bar" which passes through a hole provided in each chamber assembly. Two access ports are provided for each of the two blood-sample chambers in each assembly to allow filling with a hypodermic needle. These ports are closed after filling by means of screw plugs fabricated of nylon. A vacuum-tight seal is effected by means of an adhesive, Eastman 910. The plug screws are also sterilized with ethylene oxide. Each plug screw is long enough to project into the blood-sample chamber, and encapsulated within this projecting portion is a 1- by 6-mm Toshiba silver metaphosphate fluoroglass dosimeter rod. Two such screws in each blood-sample chamber allow redundant dose measurement for each of the ten blood samples in the BIG-I device. After the experiment,

the blood samples are removed from the chambers by puncturing one of the "windows" with a hypodermic needle. The dosimeters are recovered by cutting open the lower portion of the plug screws.

Operating Handle Assembly (Fig. 12).—The operating handle assembly is constructed of stainless steel, carbon steel, brass, and Teflon. A Teflon and brass gland assembly (Fig. 12, items 2 and 5) provides a vacuum seal for the operating rod (Fig. 12, item 6), which is the only part penetrating the otherwise hermetically sealed housing of the BIG-I device. The maximum leak rate through this seal is 10^{-6} cc-atm (helium) per second. Starting friction for the seal assembly is from 8 to 10 lb; sliding friction is from 6 to 8 lb. Because the operating rod is of very small diameter, the force required to operate the BIG-I device in a vacuum is not appreciably different from that required to overcome friction in the seal assembly. The operating handle (Fig. 12, item 3) is attached to the operating rod by a fine screw thread which permits the handle to rotate without turning the operating rod. The handle rotates approximately 30° at both extreme positions of linear travel in order to lock the device in either the "irradiate" or "nonirradiate" positions and prevent accidental movement of the blood-sample chambers. The

movement of the handle is limited, and the handle-rod assembly is supported and confined by a slotted support column (Fig. 12, items 1 and 4). The assembly is mounted by means of four screws on the BIG-I housing after it has been leak tested.

The entire BIG-I device was designed to function even if unexpected failures occur. For example, the blood samples are protected against vacuum if the spacecraft cabin pressure is lost (venting the cabin to space is not scheduled for the Gemini III mission but is for later missions and could happen accidentally, without aborting the mission, during any flight) by the sealed BIG-I housing. Even if the housing should fail, however, the blood samples would still be protected by the vacuum-tight blood-sample chambers. Similar considerations went into the design of other parts of the device. Great care was also exercised in designing a simple, hence dependable, device and also in assuring that the device could not in any way endanger the mission itself.

B. INSTRUMENTATION

The instrumentation for the BIG-I device is incorporated into a "package" designed to make use of the space available, because the control blood-sample holder can occupy a fixed position (see Figs. 7 and 8). The instrument package is held in the housing by lands which fit into the control blood-sample-holder grooves; it thus rests directly on the control blood-sample holder and serves to prevent it from moving in the grooves. The external form of the device is shown in Fig. 6 (item 6). The internal construction is shown in Fig. 13. The instrumentation provides separate records of the temperature vs time, the position of the operating mechanism vs time, the total time spent above and below the tolerable temperature range, and the ambient radiation.

The "clocks" used as time bases for the temperature and position records are Curtis Instruments, Inc., model 150 and model 120 microcoulometers (Fig. 13, Cl-4). This device is simply a fine-glass capillary tube consisting of a mercury column which is interrupted by a small bubble of electrolyte. When a voltage is applied across the column, the mercury is electroplated across the electrolyte gap at a rate determined by the current. The position of the gap is therefore an accurate measure



Fig. 12. BIG-I Operating Handle Assembly Components.

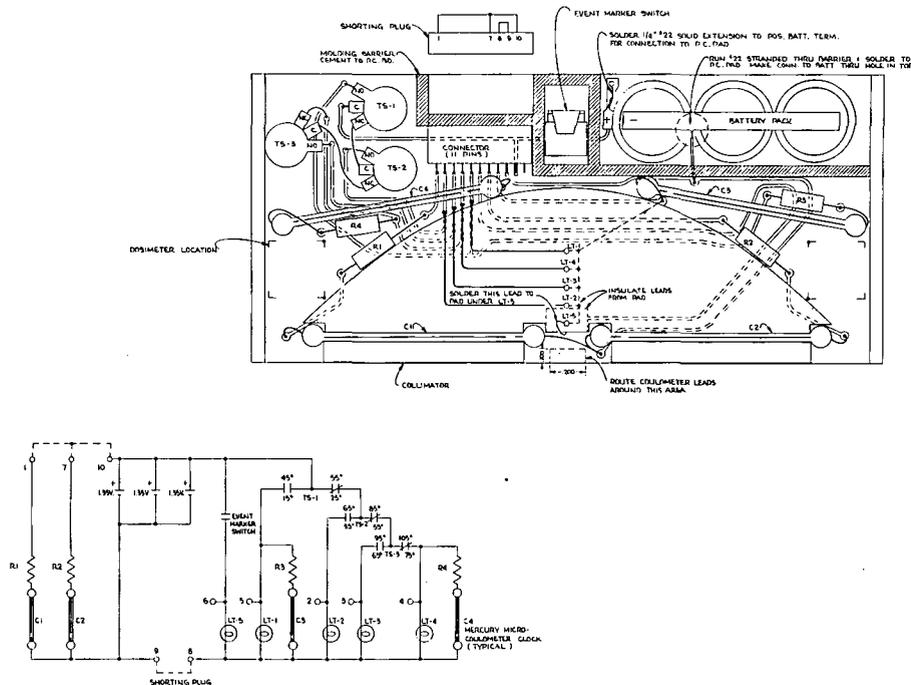


Fig. 13. Schematic Diagram and Plan of the BIG-I Instrument Package. See text for description.

of time. The temperature and position records are "written" through this transparent gap in the otherwise opaque column, by means of colored light, on strips of Ansco T-100 color reversal film. A series of five colored Kay Electric Company type 13-7 "pinlites" (Fig. 13, LT-1 through 5) are used to supply the light. The lamps are switched on and off by a series of six Texas Instrument Company model 4BT-2 thermostats (Fig. 13, TS-1 through 6). An "event" switch, mounted on the side of the instrument package, switches the position recording lamp on and off by means of a phosphor bronze spring switch leaf which contacts the side of the nearest blood-sample holder when it is in the "nonirradiating" position. The lamps are located in a Lucite "light pipe," the rear surface of which is parabolic and coated with a diffuser-reflector layer of aluminum paint. The light pipe is optically coupled to two coulometers through slits in such a way as to illuminate the coulometer gaps no matter where they are in the mercury columns. The coulometers are in turn coupled to another Lucite light pipe which carries the light to the film strips. In order to prevent reflections from

the surfaces of the mercury at each side of the gaps from degrading the time resolution of the recording, the coulometer-to-film light pipes are constructed of thin laminae of Lucite separated by thin opaque layers; they thus function as a sort of collimator. To prevent an unacceptable degree of fogging of the film, it is enclosed on five sides by a platinum shield which also serves as a light shield (not shown in Fig. 13). Two separate film records are made to ensure against loss of the record through an accident in processing. In addition, two more coulometers are connected to the circuit in such a way as to be driven only during such periods as the temperature should be above or below, respectively, the temperature range which can be tolerated by the experimental material. Thus, even if the film records are lost, there will be a method of determining whether (and for how long) the temperature limits were exceeded.

The power to drive both the coulometers and the lamps is supplied by a set of three Mallory RM 575 mercury batteries. The batteries are connected in parallel so that failure of individual units will not prevent the device from operating. The

coulometer current is limited by resistors (Fig. 13, R1-4) to give a gap movement of 1 in. per 24 hr. At this speed the coulometer gap width (0.020 in.) gives a time resolution of about 30 min. The thermostats are set to switch (and thus record) at 7.2, 12.8, 18.3, 29.5, 35.0, and 40.6°C as the temperature rises, and at 23.9, 18.3, 12.8, 1.7, -3.9, and -9.5°C as the temperature falls. These switch points were chosen to give reasonable temperature resolution in the temperature range that may be encountered during the flight.

The graphite-loaded EPON 8150 epoxy compound with TETA curing agent, used to "pot" the components (except for the batteries), is opaque and black, thus sealing the optical system against stray light. The film strips are inserted through a hole in the platinum shield; the hole is then closed with a threaded plug, thus completing the light tight assembly. The necessary wiring for the device is in the form of a printed circuit board, to which the individual components are soldered. A Cannon MTA 50-mil, 11-contact strip connector is incorporated into the package to provide external access to the circuit for testing and for "setting" the coulometers. The batteries are "potted" in place in the package with Silastic 140 silicone rubber so that they may be readily replaced. The two ambient radiation dosimeters, 8 × 8 × 4.7 mm Toshiba silver metaphosphate fluoroglass blocks, are also incorporated directly into the package. Wherever possible, components selected for the instrument package were standard commercial types which had already been tested to meet requirements for spacecraft equipment.

C. SUPPORTING EQUIPMENT

In addition to the BIG-I device itself, the successful execution of the experiment requires a number of items of special supporting equipment. In many cases, this equipment has had to be developed specifically for the purpose. Insofar as possible, everything necessary for the completion of the experiment has been kept separate from, and independent of, whatever facilities and equipment may be found to be available at the launch and recovery sites. Thus, whatever is available from other sources in the field will serve as backup for those things which have been provided specifically for the BIG-I experiment.

All operations at the launch site will be carried out in a specially equipped laboratory and assembly facility which has been built into a 38-ft van-type trailer provided by the Biology Division. This piece of equipment is shown in Fig. 14. The unit is built to operate completely independently of any external services and facilities. While it may be operated connected to external electric, water, and sewer service lines, it does not require such connections. The unit includes a gasoline-powered motor-generator set to provide electric power; water, gas, and sewage tanks; and a compressor and vacuum pump. It is air-conditioned, with absolute filtration of the air to remove airborne contaminants. Figure 15 shows the compartment which houses the air-handling equipment, motor-generator, and similar service equipment. Supply tanks and other equipment are located under the trailer (Fig. 14). The biological laboratory facility (Fig. 16) is equipped with refrigerator, CO₂ incubator, autoclave, still, centrifuge, and all normal laboratory services. Another compartment (Fig. 17) contains all necessary facilities for the assembly, welding, and leak testing of the BIG-I devices, as well as radiation detection equipment for testing and controlling radiation from the ³²P sources. The trailer is provided with complete communications equipment to link it with both private and commercial communications channels at the launch site.

The trailer will be moved to Cape Kennedy about ten days before the scheduled launch date. It will be accompanied by a well-drilled crew, including two biologists, two engineers, a specially qualified welding expert, and a radiological physicist. All these personnel have been chosen and have already begun to drill for the operation. Already two complete "run-throughs" of the experiment have been made. About 4 hr before the launch is scheduled, blood samples will be drawn from two people (one of the biologists and one of the engineers assigned to the launch site) and placed in the sterile blood-sample holders. The BIG-I devices (the "flight item" and the "control") will be welded shut, using the special water-cooled fixtures and modified welding apparatus which have been developed for this project (see Fig. 17). The devices will then be tested with the leak tester installed in the trailer, and leaks, if any, will be rewelded. After completion of the experiment, the "ground control" BIG-I device

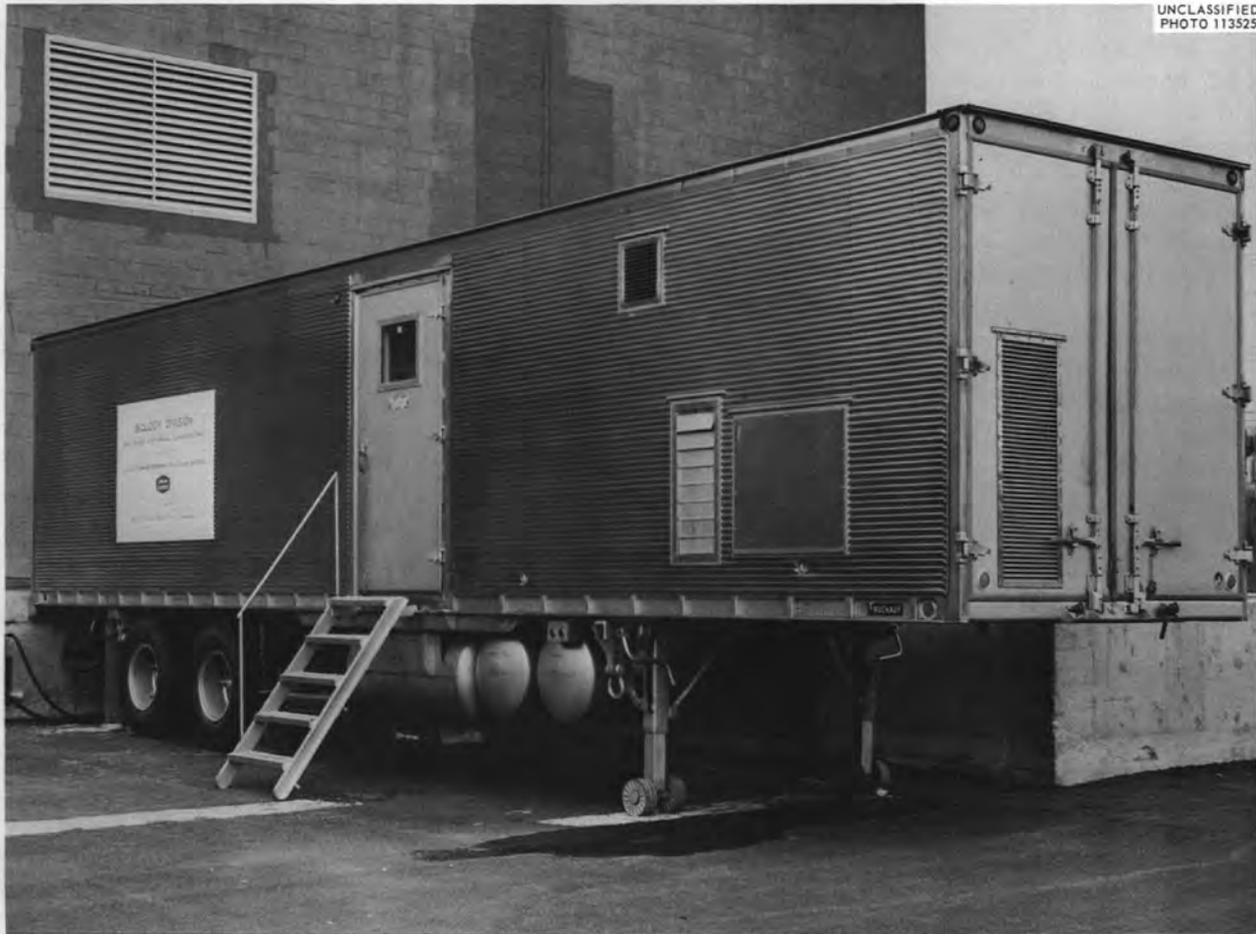


Fig. 14. Exterior View of the Specially Equipped Trailer Which Will Be Used as the Launch Site Laboratory and Assembly Facility.

will be opened in the trailer with a specially developed tool, and the blood samples will be placed in culture in the laboratory facilities of the trailer. All the remaining operations, until the microscope slides have been prepared, will be carried out in this facility.

Special "field kits" have been developed for the personnel who will be aboard the recovery vessels. Each kit contains all the necessary reagents and

sterile glassware for culture of the blood samples from the recovered "flight experiment" BIG-I device. They also include a portable CO₂ incubator, a clinical centrifuge, and a special device for opening the BIG-I housing. The recovery-area personnel will also be equipped with radiation monitoring devices to ensure against improper handling of the radioactive ³²P sources from the BIG-I device. Drilling of personnel for the three recovery sites is also already under way.

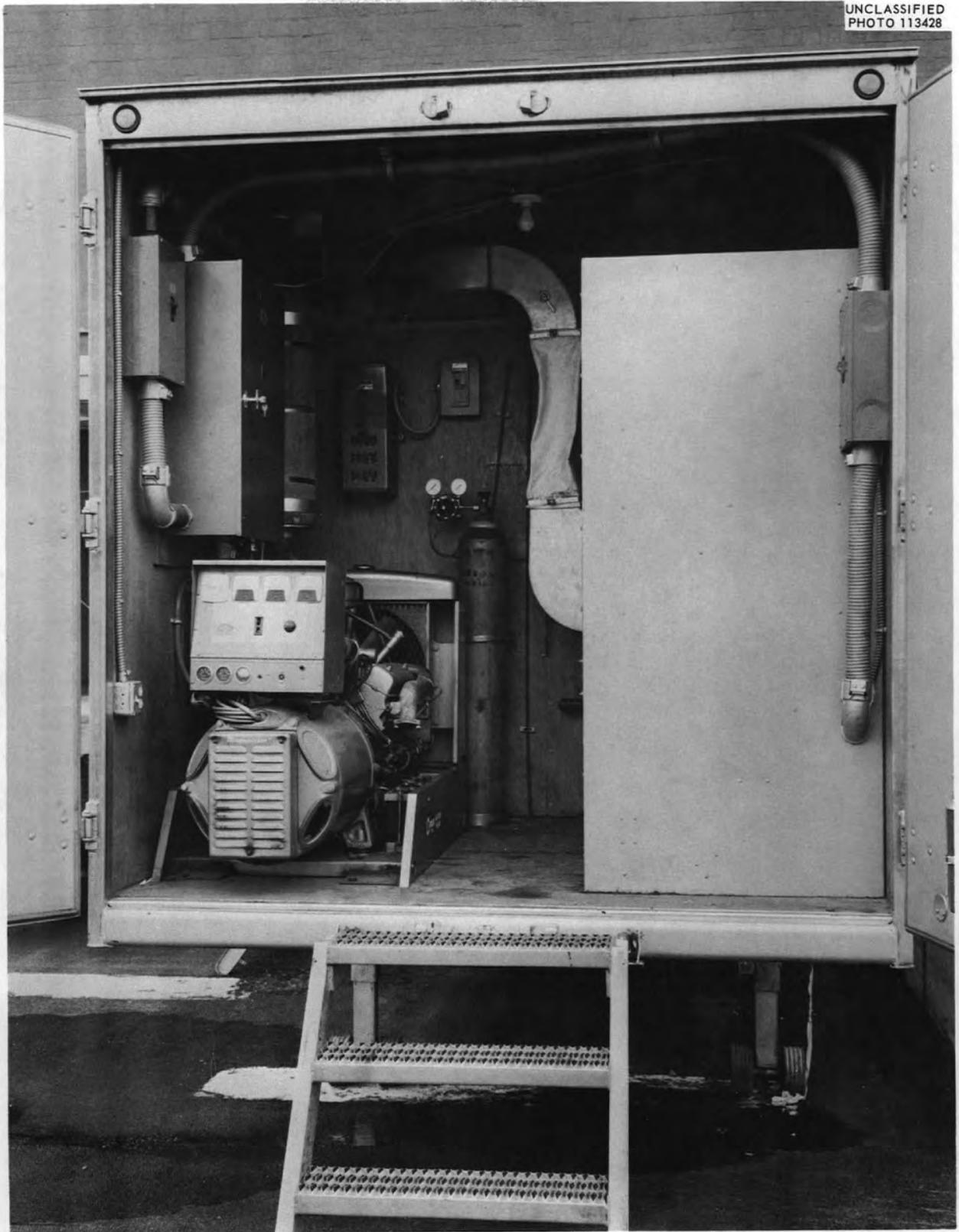


Fig. 15. Front Compartment of Trailer Showing the Motor-Generator, Air Handling, and Other Equipment.



Fig. 16. Laboratory Compartment of Trailer, Showing Incubator, Refrigerator, Centrifuge, Autoclave, and Still.



Fig. 17. Shop Compartment of Trailer Where BIG-I Devices Will Be Assembled and Tested. Note welding apparatus in foreground.

V. Testing, Qualification, and Documentation

Testing of components and assemblies for the BIG-I device began before a complete device had been assembled. The results of these tests frequently formed the basis for the original selection of a particular material or design. Subsequent repeated testing has led to several modifications, most very slight but several more substantial, such as the improved epoxy blood-sample holders adopted after extensive development work and biological testing. In general, these modifications have not changed any important external characteristics of the BIG-I device, such as its dimensions or mass.

Complete testing and qualification of the device for inclusion as spacecraft equipment aboard Gemini III were required by the NASA. Many of the tests are designed to demonstrate that the BIG-I device presents no hazard to the spacecraft, its occupants, or the successful accomplishment of the flight objectives. In addition, many tests are designed to demonstrate that the experiment will survive the space flight in good condition and will thus have the maximum chance of yielding the desired data.

A. PHYSICAL

The series of physical tests of the complete device which are required to qualify the BIG-I device as spacecraft equipment include most of the tests to which individual assemblies and components were subjected earlier. A description of the qualification testing completed thus far will indicate the scope of the physical testing program. The factors investigated include:

- 1—Temperature
- 2—Pressure
- 3—Shock
- 4—Acceleration
- 5—Vibration
- 6—Radio interference

A device assembled from randomly selected parts from a production run of five devices was

used, complete with a full set of ^{32}P beta sources with an initial activity of 25 mc, and with the blood-sample holders filled.

1. Ambient Temperature—Ambient-pressure—Temperature-pressure Combination.—The device was placed under an aluminum plate, which was heated by an electric coil. Temperature was measured by a Chromel-Alumel thermocouple located between the plate and the device. The entire apparatus was placed inside a bell jar and attached to suitable vacuum equipment. The test setup (before the heater, thermocouple, and bell jar were in place) can be seen in Fig. 18.

The pressure in the bell jar was pumped to less than 10^{-5} torr while the equipment was at room temperature (23.3°C). The pressure was then allowed to rise to 200 mm Hg. The temperature was raised to 43.3°C for 20 min while the pressure was dropped to less than 10^{-5} torr again. The operating handle of the BIG-I device was actuated. Pressure was allowed to return to 200 mm. The temperature was raised to 93.3°C for about 10 min while pressure was again decreased to less than 10^{-5} torr. The temperature and pressure were allowed to return to ambient. The BIG-I device was then placed in the freezing compartment of an ordinary refrigerator, and the temperature dropped to -12.2°C . No damage could be seen, and the operating handle of the device could be activated after all tests were completed.

Upon completion of all the other testing described in this section, a fitting was mounted in the side of the BIG-I device, and it was leak tested with a Veeco leak detector. The test setup is shown in Fig. 19. The inside of the device was pulled down to vacuum, and the device was checked for leaks by flowing helium over it from a small probe. No leaks were detected. The process was then reversed by pressurizing the device to 38.5 psig with helium and again checking for leaks. None were found.

2. Shock.—The BIG-I device was mounted in a special drop-test instrument as shown in Fig. 20. The package was attached to the instrument with tape. One drop was made in each direction along

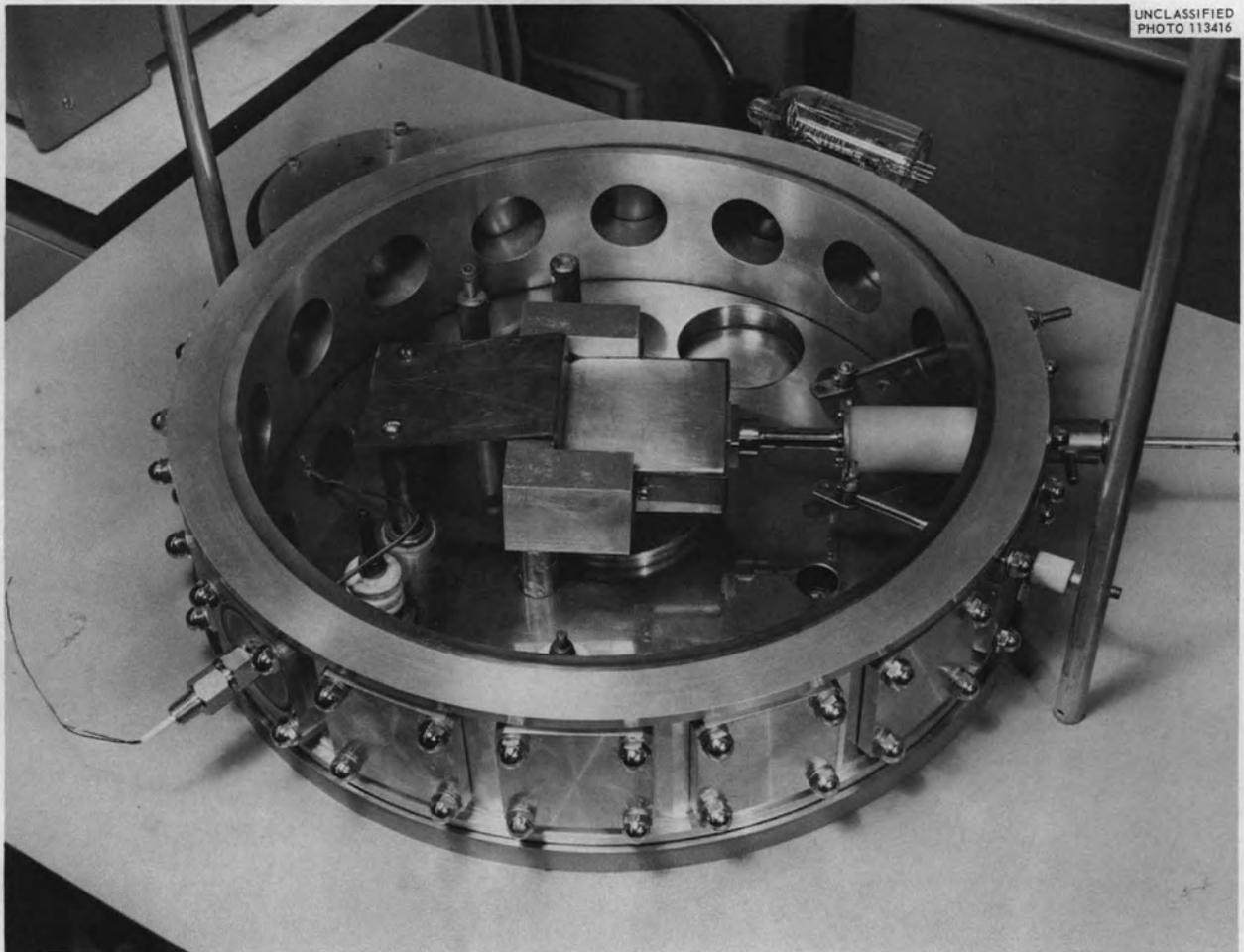


Fig. 18. BIG-I Device in Pressure-Temperature Test Setup.



Fig. 19. BIG-I Device Attached to Leak Tester to Check Integrity of Welded Joints.

each axis. Test data, including oscilloscope traces and their analysis, were recorded and analyzed. Failed amplitudes of about 55 *g* and maximum amplitudes of over 60 *g* were achieved in each instance. Upon completion of the shock tests, a visual inspection was made. The operating handle was activated and later returned to its "off" position. No damage to the device could be seen, and the operating handle worked smoothly.

3. Acceleration.—The BIG-I device was mounted in a centrifugal accelerating device as shown in Fig. 21. It was accelerated at 16 *g* for 30 sec along each of its three axes. Upon comple-

tion of the acceleration test, a visual inspection revealed no damage. The operating handle was activated and later returned to its "off" position, and was found to work satisfactorily.

4. Vibration.—The vibration test was performed in accordance with curves I, II, and III of the McDonnell Aircraft Company (the Gemini III spacecraft contractor) document MAC-8610, which outlines criteria for acceptance of Gemini III equipment. The test setup is shown in Fig. 22. The device was held to the table with tape gummed on both sides. Testing was performed along three axes by scanning from 0 to 2000 cps and detecting

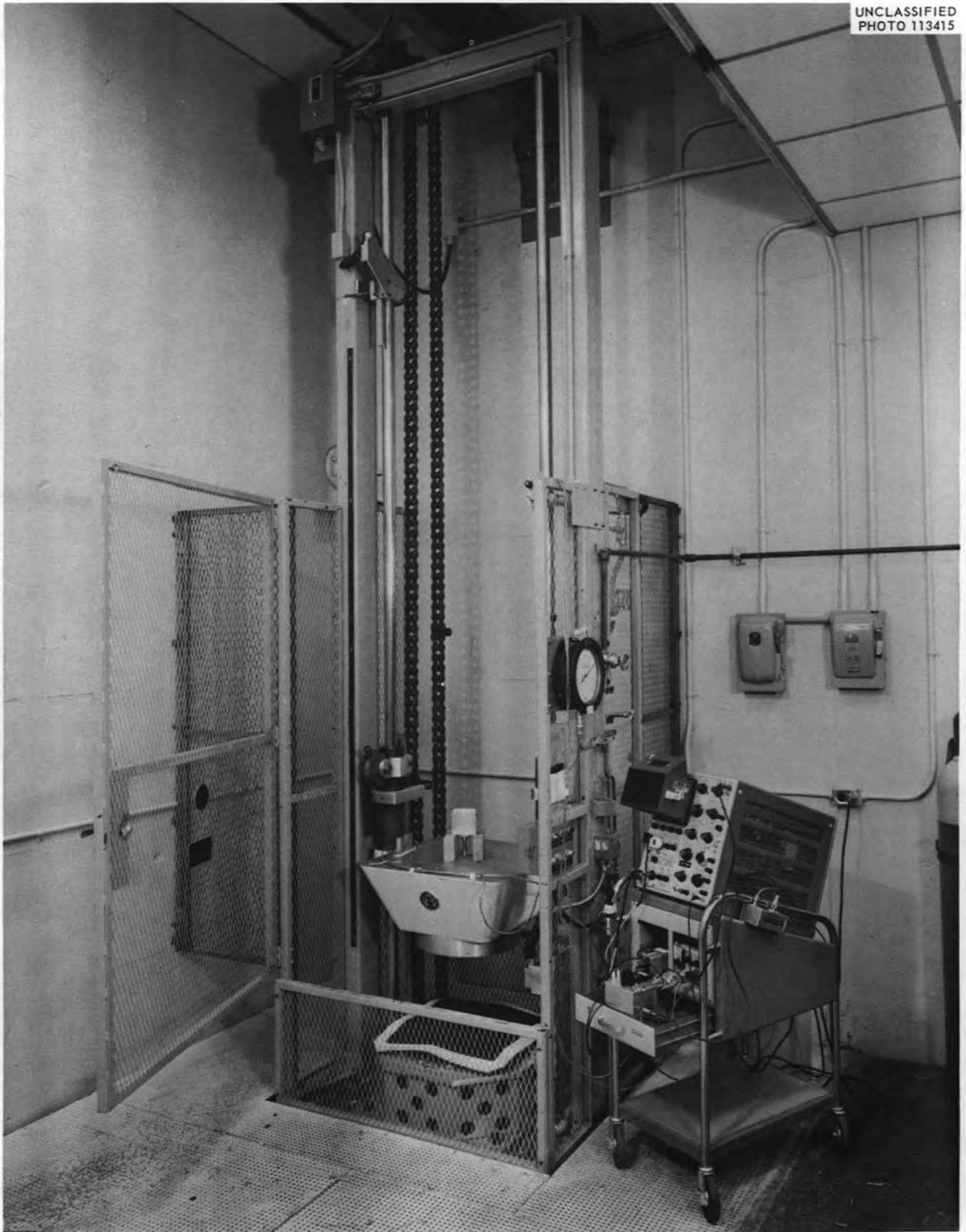


Fig. 20. BIG-I Device Attached to Shock Testing Apparatus for Qualification Tests.

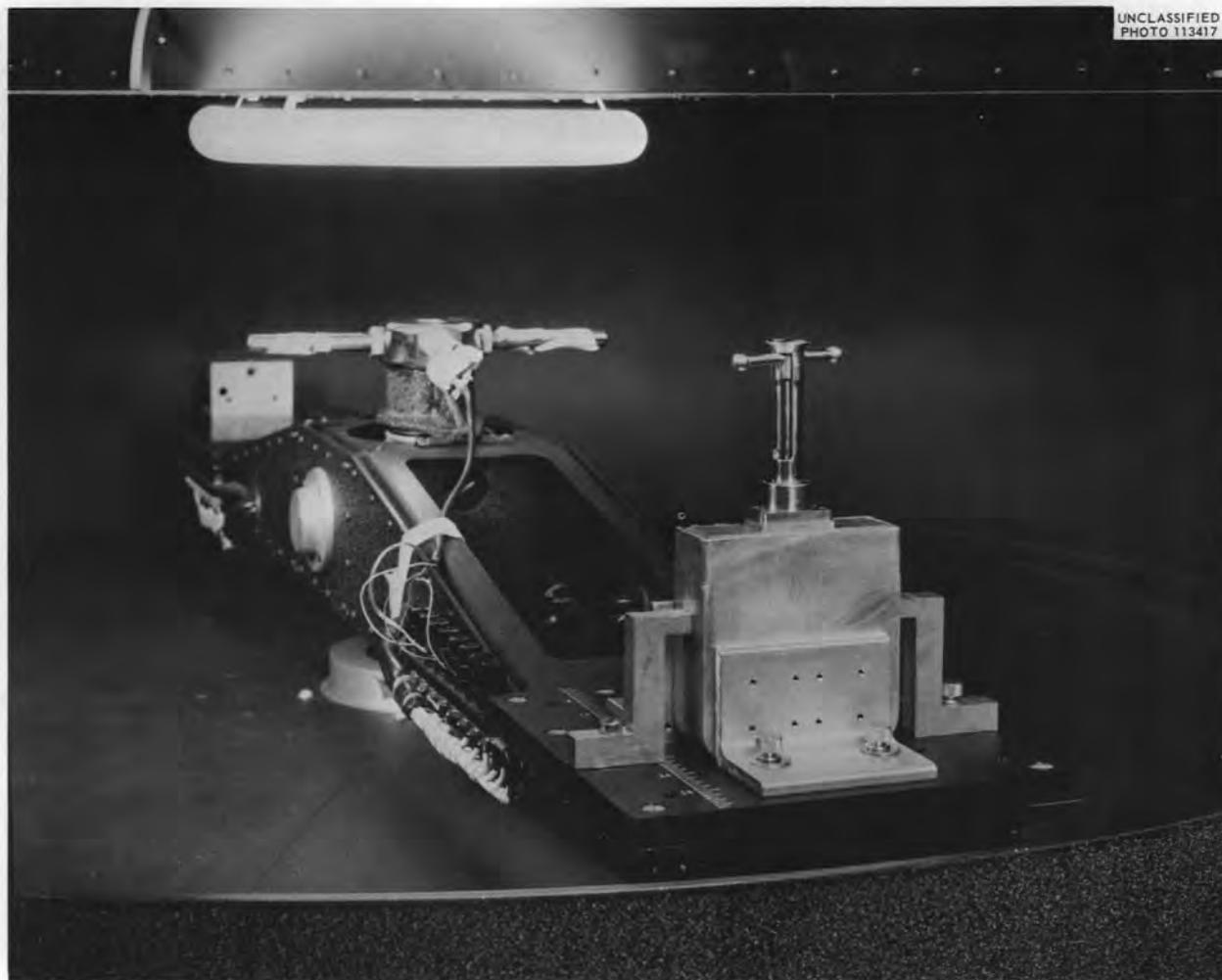


Fig. 21. BIG-I Device Attached to Centrifuge for Acceleration Test.

three resonant frequencies along each axis. The device was then vibrated for 10 min at each resonance on each axis as follows:

Axis	cps	Amplitude (g)
Upright	270	3.0
	1210	16.2
	1725	17.4
Broad side down	282	3.0
	1205	17.0
	1720	17.5
Narrow side down	290	3.0
	1220	17.8
	1735	16.9

Upon completion of the vibration test a visual inspection of the device revealed no damage. The operating handle was activated and then returned to its "off" position; it was found to work smoothly.

5. Radio Interference.—The temperature recorder module of the BIG-I device consists of a 1.35-v battery source, snap-action thermostat switches, resistors, and coulometers. The module was placed on a 0.025 in. \times 3 ft \times 4 ft copper ground plane which was bonded to a commercial, approximately 12 \times 20 \times 8 ft, copper-clad, shielded room, with its shield connected to four ground stakes embedded approximately 20 ft in the earth. All sections are electrically connected by 1/2-in. stranded copper cable. The inside has carbon-impregnated, foam-rubber pyramids covering the walls and ceiling. The ground plane was bonded as per MIL-I-26600 (USAF) paragraph 4.3.2 and Fig. 16. The tests were performed in accordance with paragraph 4.3.2. No line sta-

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Fig. 22. Vibration Test Setup with BIG-I Device Mounted on Transducer in Foreground.

bilization network was required, because the device contained its own power source.

The thermostats were activated by placing dry ice on the device. This caused the thermostats to cycle through all possible conditions; then the temperature was again permitted to rise to ambient conditions of approximately 35°C. The event switch was operated five times. Broadband and pulsed cw interference were measured at test frequencies of 0.15, 10, 20, 30, 120, 300, and 400 Mc. The noise level from the module was undetectable at the most sensitive settings of an Empire interference meter. A resonant dipole antenna was used at 30 Mc and above, while a nonresonant dipole adjusted to 27 Mc was used for frequencies below 30 Mc. The unit met the

acceptance criteria outlined in MIL-I-26600 paragraph 4.3.2.

Upon completion of all the tests outlined, the BIG-I device was opened and examined for internal damage. It was found that the leak testing had caused the contents of several of the blood-sample holders to leak. The high vacuum had also caused some flaking of the ^{32}P source material. Although the interior of the BIG-I device will almost certainly not be subjected to high vacuum, steps have been taken to prevent these conditions in the future. To prevent leaking of blood-sample holders, all holders are now being leak tested at 4 psig before acceptance. To ensure against any flaking of the ^{32}P sources if the device should be subjected to high vacuum and if, in addition, the

housing should be accidentally ruptured, a new chemical composition and bonding for the source material is being developed. In addition, all source plates will be vacuum tested before acceptance. The posttesting examination of the device revealed no defects other than those mentioned above. Thus the BIG-I device meets the requirements for both spacecraft safety and experimental integrity. Vacuum testing of internal components prior to assembly will further increase the ability of the BIG-I device to survive extremely improbable accidents.

B. RADIOLOGICAL

A number of radiological tests of the BIG-I device have been completed. These include:

- 1—External radiation level
- 2—Phosphorus-32 source homogeneity

Several other investigations are in progress:

- 3—Fricke dosimetry
- 4—Spectral analysis
- 5—Fluoroglass dosimeter calibration

1. External Radiation Level.—A complete but unwelded BIG-I device, with ^{32}P source plates with nominal activities of a total of 25 mc (four plates each with nominal activities of 2.5, 1.875, 1.25, and 0.625 mc, for a total of 16 sources) was tested for external radiation levels. The radiation field around the BIG-I device consists of both bremsstrahlung x rays induced by the beta irradiation of the BIG-I structure and the beta particles themselves, a small fraction of which escape from the housing. Measurements were made with a Victoreen Thyac G-M survey meter, which was calibrated against a National Bureau of Standards calibrated Victoreen electrometer and 0.25-r chamber. Readings were made at a number of points in each of three planes around the BIG-I device at a distance of 10 cm, with the beta shield of the Thyac probe in place. The bremsstrahlung profiles thus determined are shown in Figs. 23, 24, and 25. It will be seen that the bremsstrahlung did not exceed 7 mr/hr at 10 cm in any direction and that it was considerably less at most points. A test for applicability of the inverse-square law was also made, and it was found that this law held for distances greater than 10 cm and could thus be

used in calculating the radiation field in the Gemini III vehicle, once the location of the device in the spacecraft was known. Tests with the Thyac meter with its beta shield removed showed that the beta-particle field around the BIG-I device was a maximum of three times the bremsstrahlung level on the "hot" side of the device. The bremsstrahlung field would be a maximum of about 0.6 mr/hr at a distance of 1 ft from the device. The BIG-I device thus does not present any radiation hazard to the spacecraft or its occupants. Further, virtually all of what little radiation there is from the device will be shielded out by the astronauts' space suits and by similarly thin spacecraft components.

2. Phosphorus-32 Source Homogeneity.—The homogeneity of the ^{32}P sources has been tested by making autoradiographs of a large number of the sources which have been made to date. Autoradiographs were made by exposing wrapped films in contact with the ^{32}P surfaces of the source-plate assemblies. High-contrast emulsion and development were used, thus magnifying local differences in radiation intensity. The results of these tests indicate that the sources are relatively homogeneous and do not vary in intensity by more than 20% except for very small local defects.

3. Fricke Dosimetry.—The absolute dosimetric determinations are being made by exposing Fricke Fe^{2+} solution in the blood-sample holders positioned between ^{32}P source plate pairs. Initially, some difficulty was experienced because the solution was somewhat oxidized by the epoxy blood-sample holders themselves. This problem has been overcome, however, by making the exposures in the cold, which slows down the rate of chemical oxidation but not the rate of the radiochemical oxidation, by means of which the dose is determined. The Fricke determinations, while not yet complete, show that the reflection of beta particles from the platinum plate assemblies contributes an additional 70% to the dose seen by the blood sample. The exact contribution of beta-particle reflection is being measured by means of specially constructed ^{32}P sources placed on virtually non-reflecting beryllium foils.

4. Spectral Analysis.—Spectral analysis of the radiation from the BIG-I device was begun, using a multichannel analyzer with sodium iodide and anthracene crystal scintillation detectors. Pre-

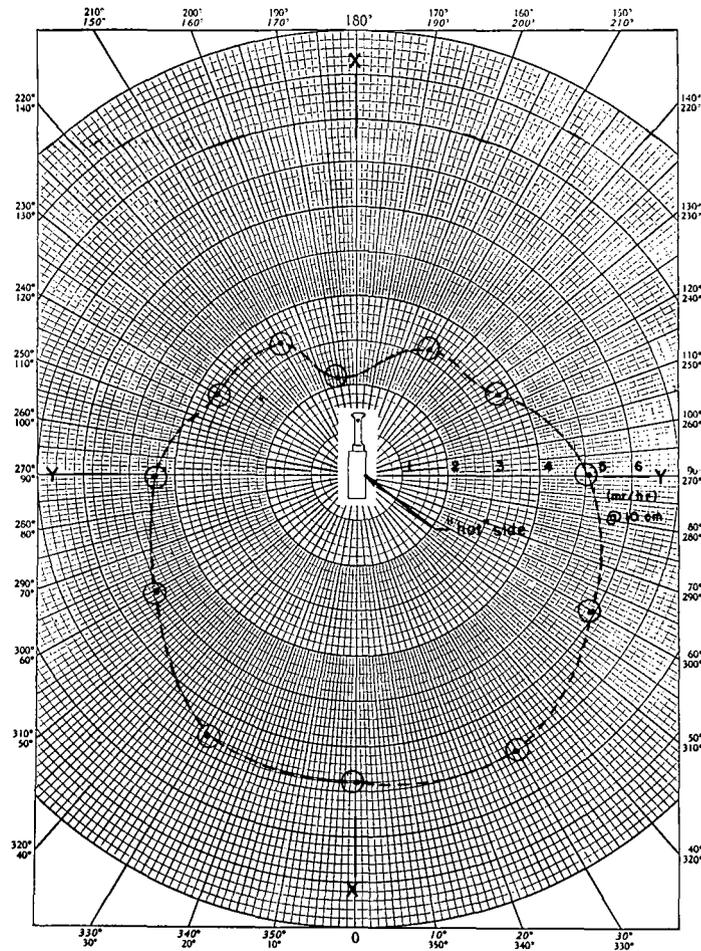


Fig. 23. Bremsstrahlung Radiation Field at 10 cm in the X-Y Plane Around BIG-I Device Loaded with ^{32}P Sources Totalling 25 mc.

liminary results show the expected spectral distributions, but detailed analysis has not been completed.

5. Fluoroglass Dosimeter Calibration.—The Toshiba silver metaphosphate fluoroglass dosimeters are read in a Toshiba ultraviolet fluorimeter. Two calibration standards are being used. A series of dosimeters have been exposed to ^{60}Co gamma rays at the National Bureau of Standards and are used as secondary standards for instrument calibration. The instrument has been shown to be both extremely linear and extremely stable. The conversion factor for reading the fluoroglass dosimeters after exposure to ^{32}P beta particles is being determined by making parallel

measurements with the Fricke dosimeter system. In addition, special 0.1-mm-thick fluoroglass plates have been obtained from the Toshiba company and are being used to determine the depth-dose curve for the 3-mm blood samples when exposed in the BIG-I device.

C. BIOLOGICAL

Biological tests of the acceptability of materials for the blood-sample holders were started along with the initial development work for the BIG-I device. Blood samples were placed in dishes with samples of possible materials for a period of 24 hr.

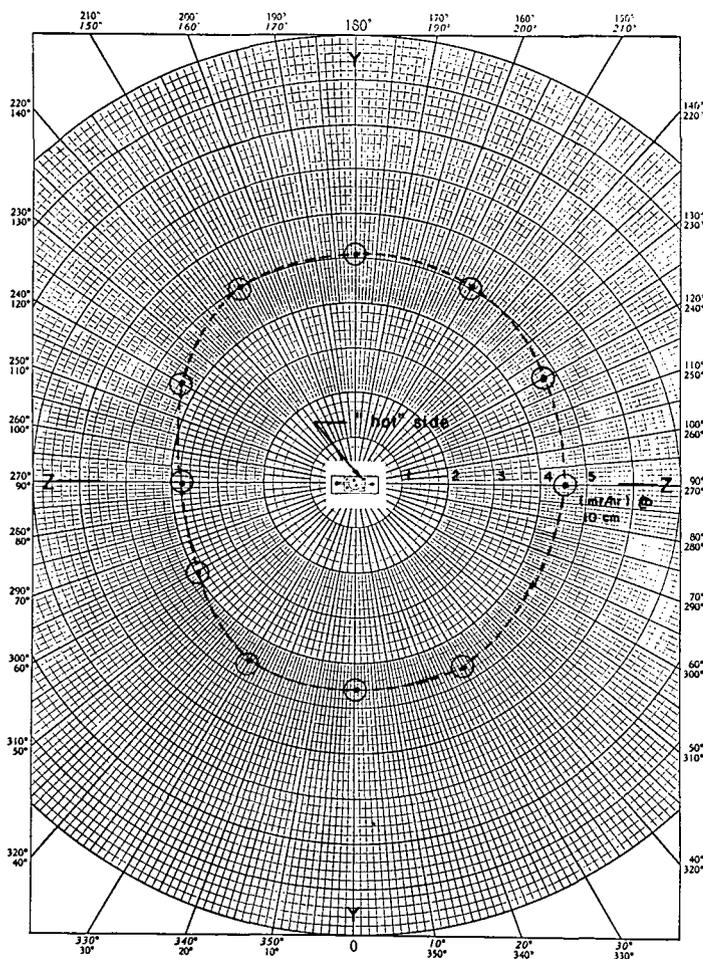


Fig. 24. Bremsstrahlung Radiation Field at 10 cm in the Y-Z Plane Around BIG-I Device Loaded with ^{32}P Sources Totaling 25 mc.

They were then placed in culture and prepared in the same manner as the actual flight material will be. Materials were rated on the basis of the number and quality of metaphase figures in the final preparations. After the first epoxy resin blood-sample holders were fabricated, they were further tested. Several sets of chambers were cleaned and ethylene oxide sterilized. Blood samples were introduced, and the filling ports were closed with nylon dosimeter screws. After 24 hr at room temperature, the blood was removed and processed as before. The results of these tests were also satisfactory.

Potential donors of blood for the experiment were also repeatedly tested for suitability. Two

"good growers" were selected on the basis of these tests, and a number of backup blood donors were identified among the personnel who will comprise the launch site crew.

Two full-scale run-throughs of the experiment have been made with the selected blood donors and with samples exposed to the ^{32}P sources in welded BIG-I devices. The first test was highly successful; all blood samples yielded excellent microscopic preparations which are now being analyzed for aberrations. The second test was also successful except for an electrical failure which caused a change in incubator temperature and reduced the number of metaphase figures in the final preparations to unsatisfactory levels. This

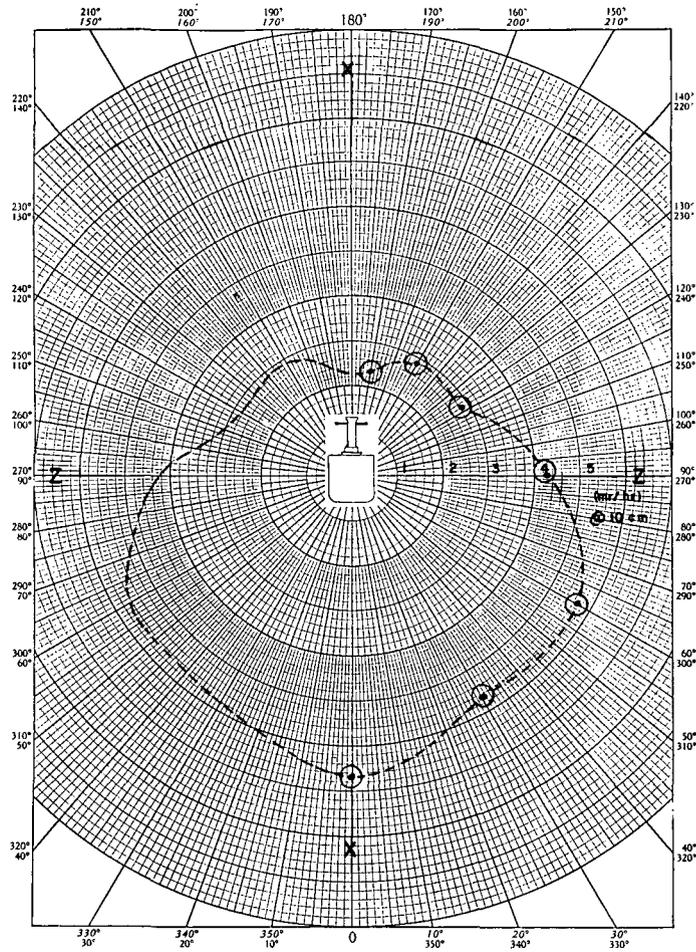


Fig. 25. Bremsstrahlung Radiation Field at 10 cm in the X-Z Plane Around BIG-I Device Loaded with ^{32}P Sources Totalling 25 mc.

difficulty emphasizes the necessity for further run-throughs of the experiment in order to detect points at which such failures might possibly occur

and thus make it possible to take steps to ensure against their occurrence during the actual experiment.



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