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NEUROSPORA EXPERIMENT P-1037

PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

COVERING THE PERIOD FROM INITIATION

THROUGH SEPTEMBER 30, 1966

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ORNL-TM-1734

Contract No. W-7405-eng-26

BIOLOGY DIVISION

NEUROSPORA EXPERIMENT P-1037

Progress Report

to the

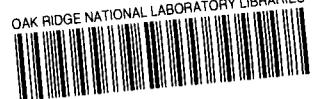
National Aeronautics and Space Administration

Covering the Period from Initiation Through September 30, 1966

1967

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I. BIOSATELLITE PROJECT OBJECTIVES

The mission of the biosatellite project is to investigate the interaction of living organisms with certain aspects of the space environment. In the three-day flight, the effect of weightlessness upon a number of biological processes will be studied with different organisms. The vehicle will carry a radiation source (^{85}Sr) which will be used to study the effects of weightlessness on such different genetic effects as virus activation in lysogenic bacteria and induction of specific locus mutations and chromosome breakage in *Neurospora*, in germ cells of *Drosophila* and *Habrobracon*, and in somatic cells of *Tradescantia*, *Drosophila*, and *Tribolium*. The effects of weightlessness upon feeding and digestion in *Amoeba*, upon cell division and early development of frog embryos, upon the orientation, biochemistry, and histochemical location of various enzymes in the developing wheat seedling, and upon orientation and biochemical composition of the leaves of growing pepper plants will also be studied.

II. OBJECTIVES OF NEUROSPORA EXPERIMENT IN THE BIOSATELLITE

With the *ad-3* forward-mutation system in *Neurospora*, it is possible to detect and distinguish different types of mutations, ranging from single base-pair substitutions, or single base-pair insertions or deletions to gross chromosome deletions. *Neurospora* conidia will be submitted to known exposures of gamma radiation ranging from less than 500 r to about 6000 r in the flight vehicle under weightlessness, as well as in a control experiment on the ground with an identical radiation source in a simulated spacecraft. Conidia will also be placed in the shielded sections (aft payload) of both the flight and control vehicles to serve as unirradiated controls. After the flight, conidia will be assayed for survival as well as for specific locus mutations in the *ad-3* region. Dose-response curves for the two experimental groups of conidia will be obtained for survival and mutation induction. Random samples of mutants from each group will be subcultured for further genetic analysis to determine whether the frequencies of the different types of mutants from the flight and ground control groups are different. These data can also be compared with similar data from preflight experiments on the ground, as well as with postflight experiments in which temperature can be adjusted to duplicate more precisely that profile experienced during flight. Records of the vibrational stresses to which the space capsule is subjected during ascent and reentry will also be available for postflight simulations.

III. LABORATORY AND GROUND CONTROL MUTATION DATA

A. Acute X-Ray Exposures

Appendix A is a paper describing the relation between total dose and *ad-3* mutant yield obtained at 2 to 6°C and high dose rate (1000 r/min). It is observed that *ad-3^R* mutations, which result from point mutation, increase in direct proportion to the dose, while *ad-3^{IR}* mutations, which result from small deletions, increase as the square of the dose. Each *ad-3^{IR}* mutant arises as the result of the interaction of two independent events, neither of which by itself would be detected in this system as an *ad-3* mutant. These events occur outside the *ad-3A* and/or the *ad-3B* locus, whereas *ad-3^R* mutations result from events within the *ad-3A* or *ad-3B* locus. Mutations of genotype *ad-3A ad-3B* increase as the square of the dose, and the frequencies of mutations of this genotype can be used to obtain a first approximation on the effect on *ad-3^{IR}* mutations in general. This paper is also useful in that it provides a concise description of experimental procedure and of the various strains used in the forward-mutation experiments as well as in the subsequent genetic analyses.

B. Chronic Radiation Exposures

Since irradiation during the entire three-day biosatellite mission seemed desirable to study the effect of weightlessness, a low-intensity source was planned. However, to obtain the appropriate background data it was necessary to determine whether the dose-response curve for low-intensity, long-term x irradiation is different from that for 1000 r/min rate and also to determine whether there was any effect of temperature (21°C in the spacecraft vs 2 to 6°C used above) or postirradiation storage. In an experiment designed for this purpose, conidia were collected on Millipore filters and irradiated at room temperature with total exposures of approximately 5000, 10,000, 20,000, and 40,000 r in each of the following three different ways: (1) at a dose rate of 10 r/min; (2) at a dose rate of 1000 r/min, followed by prompt plating and jug inoculation; and (3) at a dose rate of 1000 r/min, with an incubation period of 66 hr between irradiation and inoculation.

The survival of heterokaryotic conidia was much higher at all doses with 10 r/min than at 1000 r/min, so that a dose-reduction factor (DRF) of about 2.6 is observed. Also the frequencies of *ad-3A ad-3B* double mutants (which are virtually always small deletions) are much lower at the lower dose rates and show an identical DRF. Our interpretation of the incomplete data from this experiment is that at low dose rates (like those in the biosatellite) there is considerable repair of those events producing chromosome breakage but not alterations in the DNA, so that the majority of mutations obtained will be point mutations in the *ad-3A* or *ad-3B* locus. This interpretation is based upon the direct proportionality between dose and *ad-3* mutant yield at 10 r/min in samples of mutants thus far analyzed.

C. Ground Control Data from Biocompatibility Tests

In three experiments at the Ames Research Center, ^{85}Sr and ^{137}Cs were used in prototype or flight-type hardware as radiation sources at room temperature. The dose-response curves for forward mutation obtained in these experiments are summarized in Fig. 1. It is clear that all the data can be plotted on the same dose-response curve and that the response is proportional to the dose. Furthermore, there does not appear to be a difference between vibrated and unvibrated samples.

In the biosatellite flight experiment it should be possible to look at the effect of orbital weightlessness plus other stresses of space flight both upon the process of mutation induction and on the repair of premutational damage.

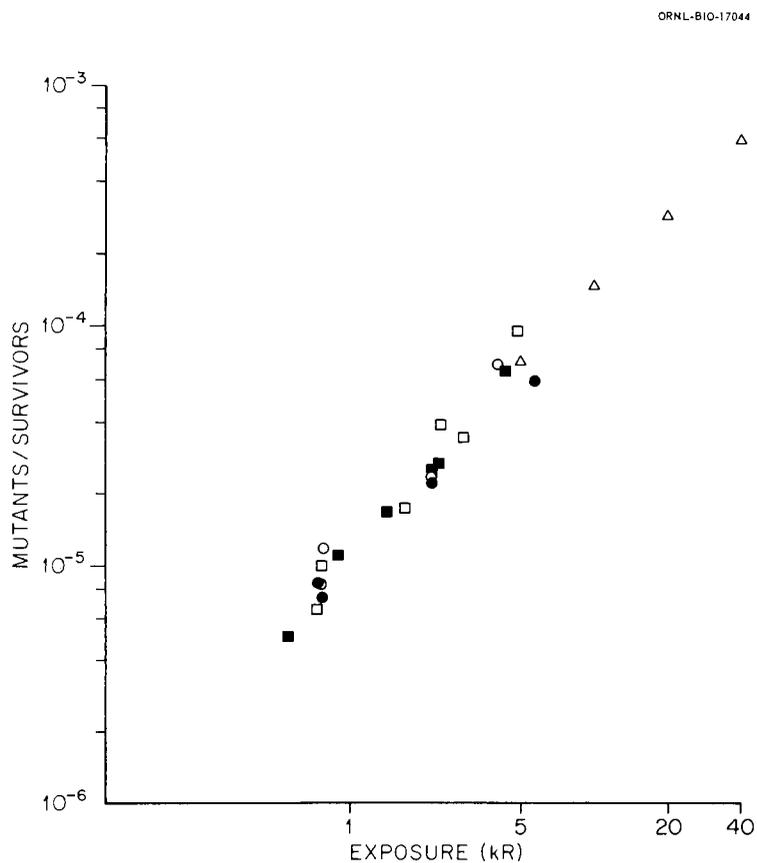


Fig. 1. Forward-Mutation Frequencies Plotted Against Exposure for the Three Radiation Experiments with Prototype Hardware at Ames Research Center. Δ 250 Kvp x rays, 30 ma, 3 mm aluminum, at 10 r/min (conidia on filters on glass beads). \bullet ^{137}Cs gamma irradiation with vibration to simulate flight profile. \circ ^{137}Cs gamma irradiation without vibration. \blacksquare ^{85}Sr gamma irradiation with vibration to simulate flight profile. \square ^{85}Sr gamma irradiation without vibration.

IV. HARDWARE DEVELOPMENT FOR NEUROSPORA EXPERIMENT

Ideally, the Neurospora package would be required to contain a large number of Neurospora conidia under sterile, nontoxic conditions with a sufficient supply of air so that they do not become anaerobic during flight. It was decided that conidia deposited onto moist Millipore filters could be held tangentially to the isodose lines on screens which would permit free exchange of air between the conidia and the box which would surround the screens and preserve both sterility and high humidity. All materials used would need to be autoclavable, nontoxic, and capable of causing minimal radiation shielding and backscattering.

Plans, description, and instructions for the prototype packages are found in Biosatellite Program Document Number 65SD460. When these were actually used in biocompatibility tests, difficulties and defects in design were noted, and the remedies which were recommended at that time (May 21, 1965) are listed in Appendix B.

The following alterations in design were then executed: (1) The design was altered so that only one type of housing, with no difference in base plate design, would fit into any of the five Neurospora positions in the vehicle. (Previously a package with one type of base plate was required for positions on the radiation shield and in the unirradiated position, and another type was required for positions on the bracket.) (2) The modules were modified in such a way that one face plate was removed and the module fastened to the housing cap. The housing cap was also altered to screw into the housing, so that the springs, which had distorted badly during autoclaving, were no longer required. (3) The cap was knurled so that it could be grasped readily and screwed firmly into place in the housing. (4) The polypropylene screens were fastened more firmly to the disks and the edges trimmed better. (5) The new barriers were either thickened or fabricated from a stiffer material and were more easily handled and installed than the old limp ones. (6) The old sponge holder and the hole in the housing required to accommodate the sponge holder were eliminated. For preserving high humidity in the package, a new ring-shaped sponge was provided which encircled the module at about the center. These rings were badly designed and were never used, but were replaced by a cylindrical sponge located in a space in the housing cap. (7) The design of the lithium fluoride dosimeter tube holders was also much improved.

Figures 2 to 5 are photographs of the modified Neurospora flight assemblies, both assembled and disassembled. We have been advised by the fabricators to autoclave the modules in the assembled condition so that the disks will not warp. This is a minor inconvenience, but it is not an impossible task. The major structural weakness in the hardware is in the long screws; a number of these have been broken off at the threaded portion, even though a calibrated torque-limiting screwdriver is used for assembly. No other difficulties have been encountered, and broken screws can be replaced from spare hardware.

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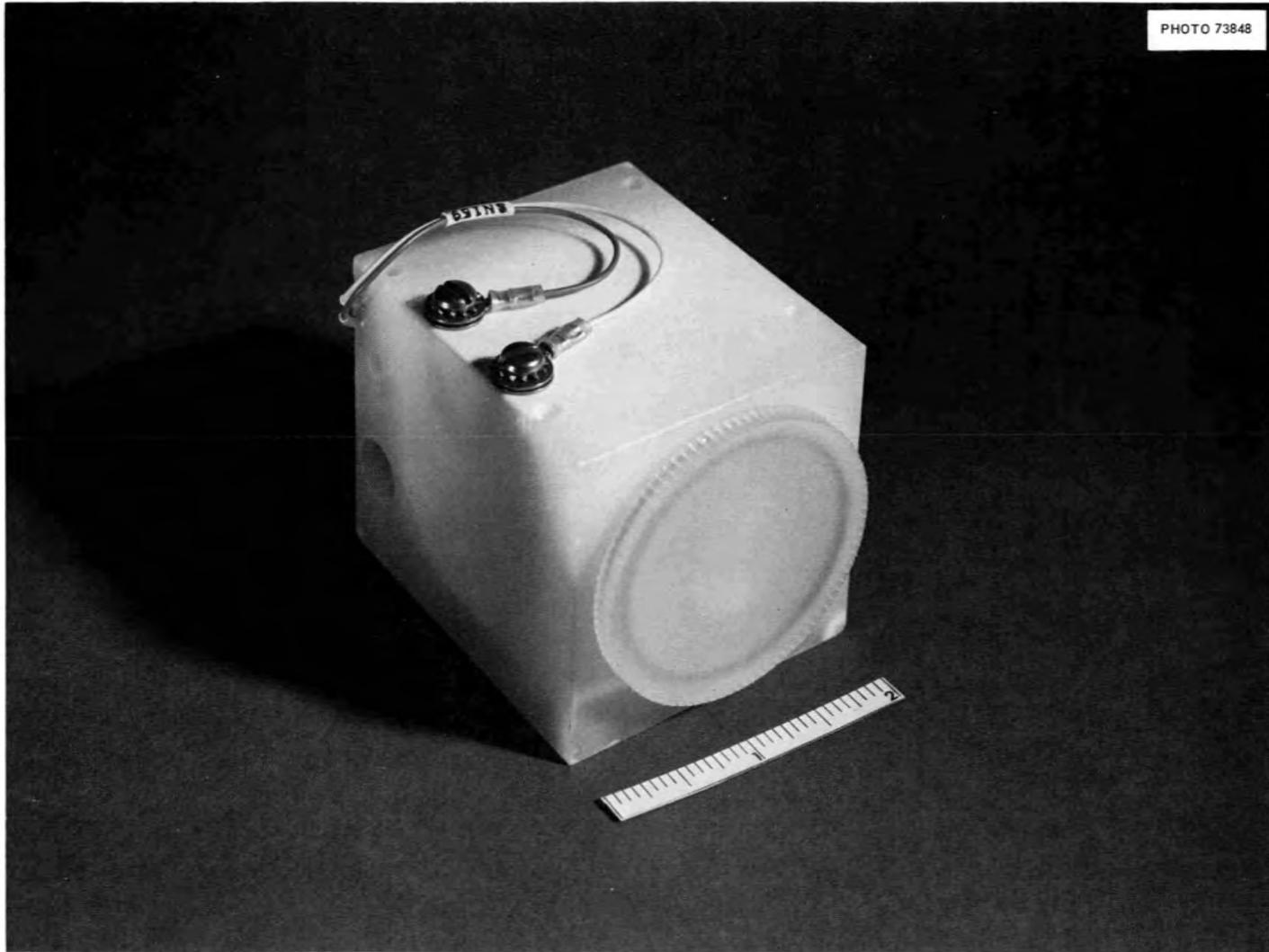


Fig. 2. Photograph of Neurospora Flight Assembly Showing Assembly Cap at Front and Thermister Levels.

PHOTO 73847

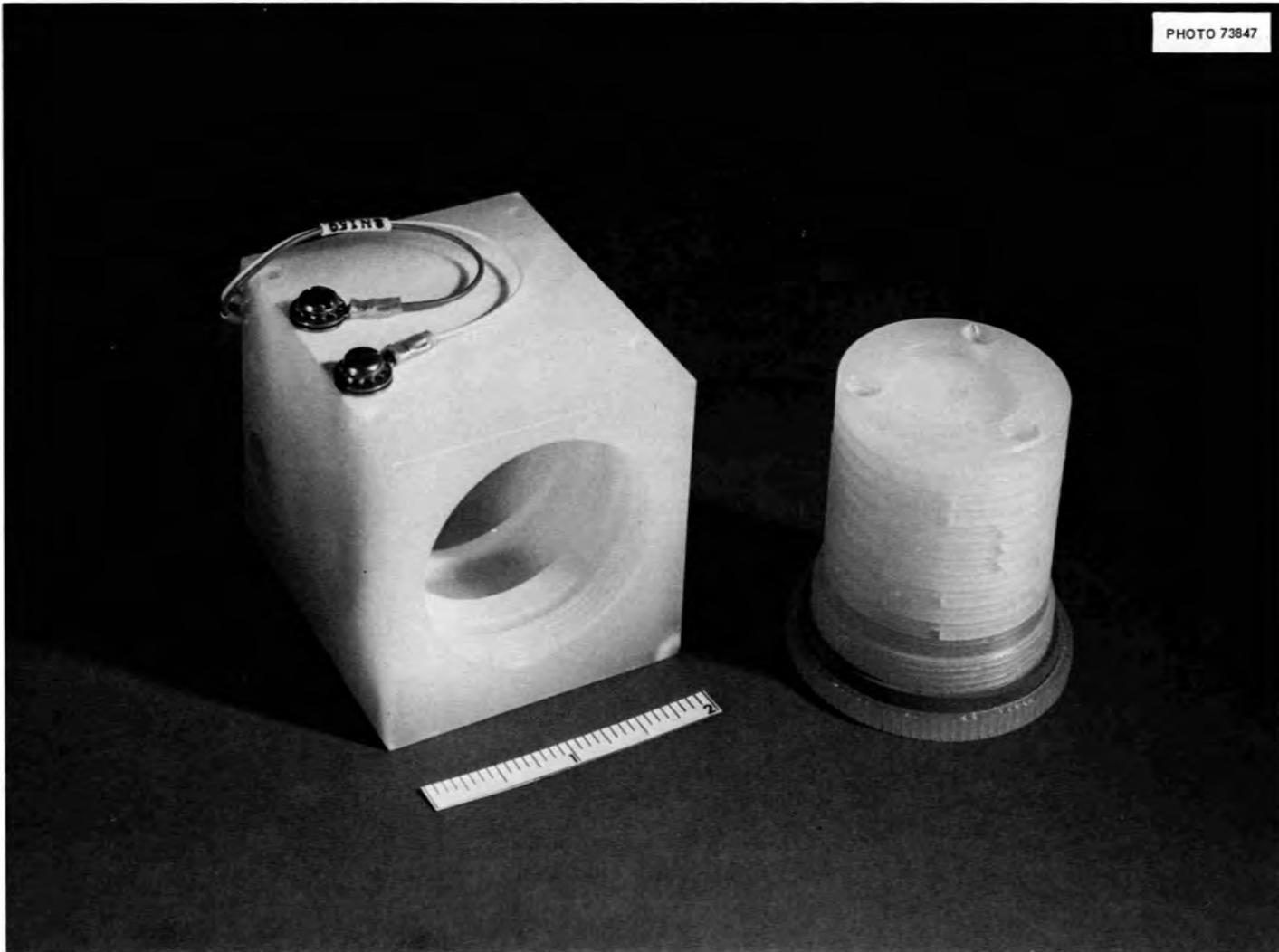


Fig. 3. Photograph of Separated Neurospora Flight Housing and Module.

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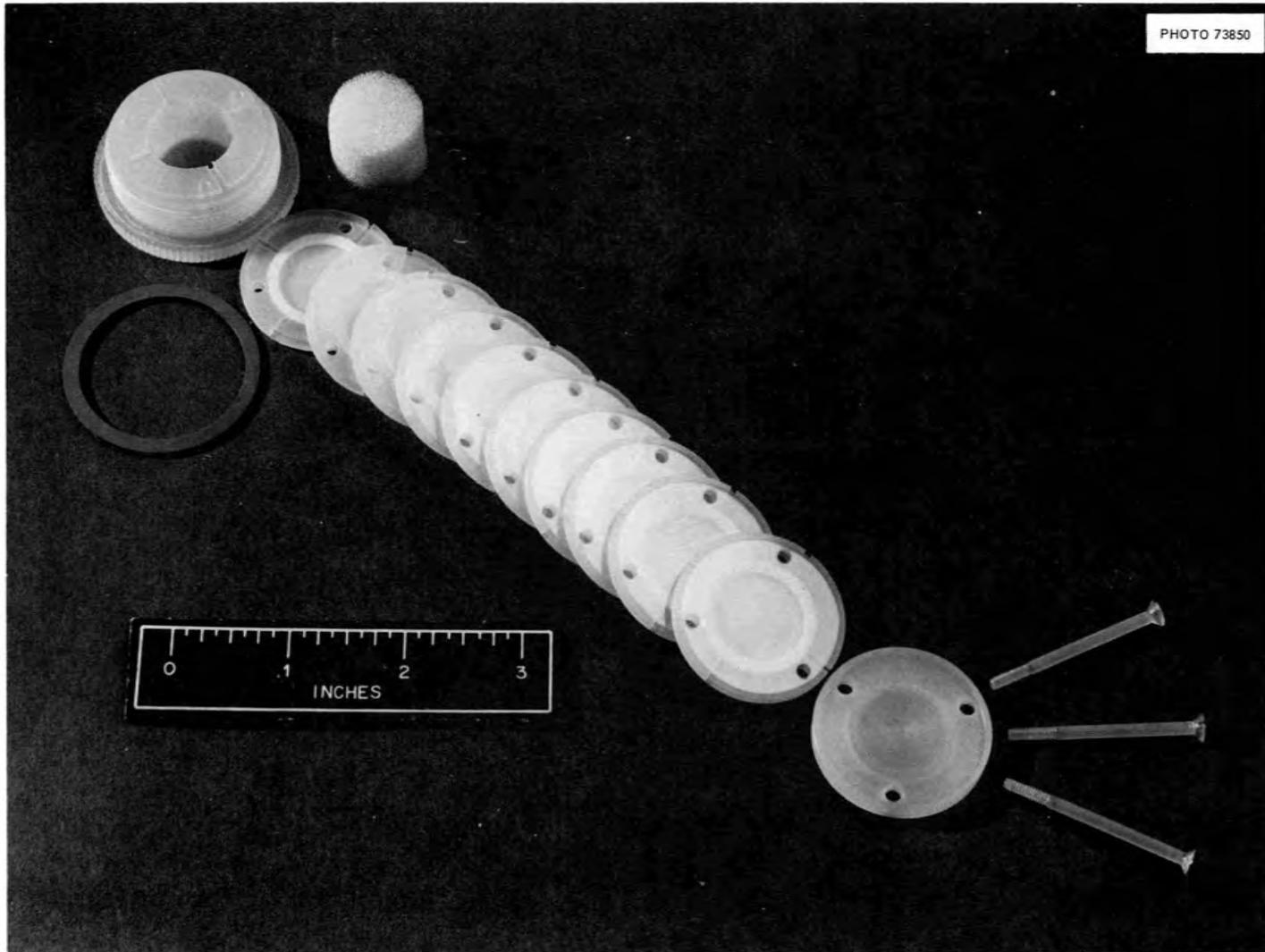


Fig. 4. Photograph of Disassembled Module Showing Gasket, Assembly Cap, Sponge, Ten Filter Disks, End Cap, and Screws.

PHOTO 73849

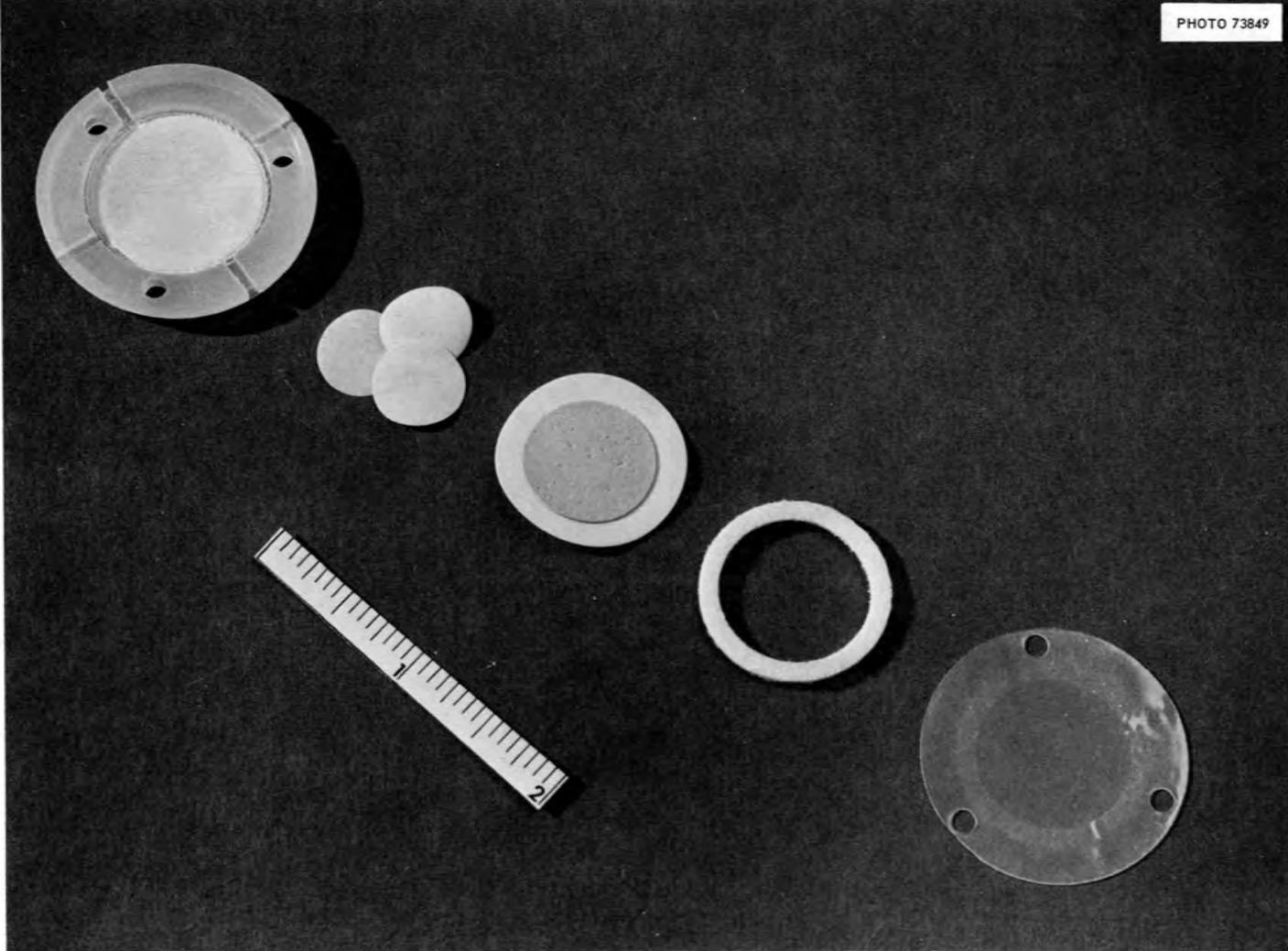


Fig. 5. Photograph of Disassembled Filter Disk Showing Filter Ring, Three Lithium Fluoride Teflon Disk Dosimeters, Millipore Filters, with Deposited Conidia, Retainer Ring, and Barrier.

V. DOSIMETRY

A. General

The radiation source will be a mass of ^{85}Sr powder enclosed in a hollow metal disk. During the 65-hr period of radiation (from about 1 hr after insertion of the vehicle into orbit until about 1 hr before reentry of the vehicle into the atmosphere), the radiation source will be exposed through a hole in the center of the circular radiation shield; at other times it will be enclosed in a heavy metal sphere. Although the radiation experiment assemblies themselves are made mainly of materials which will offer minimal absorption and scattering of ionizing radiation, the metal radiation shield and brackets used to support some of the assemblies, as well as the spacecraft wall and heat shields (aluminum and fiber glass, mainly), could cause nonrandom radiation exposures and differences in the energy spectrum at comparable distances around the source. For this reason it has been considered desirable to place dosimeters as close to the irradiated conidia as possible. Additional requirements are that the dosimeters be small and light in weight, that they be sterile if placed within the Neurospora housings, and that their scattering and absorption characteristics be not too much different from water and from the polypropylene housings and modules.

Two independent systems of thermoluminescent dosimetry will be used. (1) Ames Research Center personnel will provide samples of lithium fluoride powder in polyethylene tubing which are placed into cylindrical chambers along two edges closest to the radiation source and two edges farthest from the source. These are not sterile but are not in the chamber which contains the module and conidia. The relationship between the radiation exposures at these positions and the exposures to the conidia in the modules must be determined from preliminary radiation experiments. (2) Independent determinations of the radiation exposures by thermoluminescence readings will be made by ORNL personnel using 5-mil-thick disk-shaped dosimeters composed of lithium fluoride embedded in Teflon. These can be sterilized by autoclaving and placed immediately adjacent to the conidia on the filters in the modules. Because they have the same configurations as the layers of conidia, they should be especially useful for determining the average exposure for conidia near the source, where small uncertainties about distance result in large uncertainties about total exposure.

B. Ames Dosimetry Exercise

In conjunction with the Ames biocompatibility test, a dosimetry exercise was carried out in which flight-type modules and housings, loaded with 5-mil lithium fluoride disk dosimeters at each filter position, as well as with tubes of lithium fluoride powder at the edge positions, were exposed to ^{85}Sr gamma radiation. The housings were assembled and attached to an exact duplicate of the flight vehicle, with the radiation source in its appropriate position at the center of the radiation shield. The major purpose of this exercise was to observe whether the relationship between exposure and distance from source showed departures from normal due to either unusual

scattering characteristics of the components of the vehicle or to unexpected absorption by the housings or modules. It was also possible during this exercise to obtain data which would permit cross calibration between different systems of dosimetry (e.g., lithium fluoride powder for the Ames dosimetry group and for the Tradescantia experiment, lithium fluoride disks for the Neurospora group, and glass rods for the Habrobracon group) and comparison of exposures at different positions in the spacecraft.

In Fig. 6 are plotted the average thermoluminescence readings for this exercise. It is felt that the exercise was not entirely satisfactory, because in many cases dosimeters which were close together gave rather different readings. This is attributed to heterogeneity in sensitivity of the batch of dosimeters used for the exercise. We have recently received a new batch of dosimeters which should meet all requirements for calibration runs and prime and backup flights. These are now being evaluated for heterogeneity in calibration runs with both x rays and ^{85}Sr gamma radiation. It is possible to relate the thermoluminescence data to a calibration curve obtained with ^{85}Sr gamma radiation outside the vehicle and in this way estimate the exposure to each filter position. If one assumes normal dose-distance relationships for a point source, then the lines in Fig. 6 can be transferred to another scale (Fig. 7) based on exposure rather than

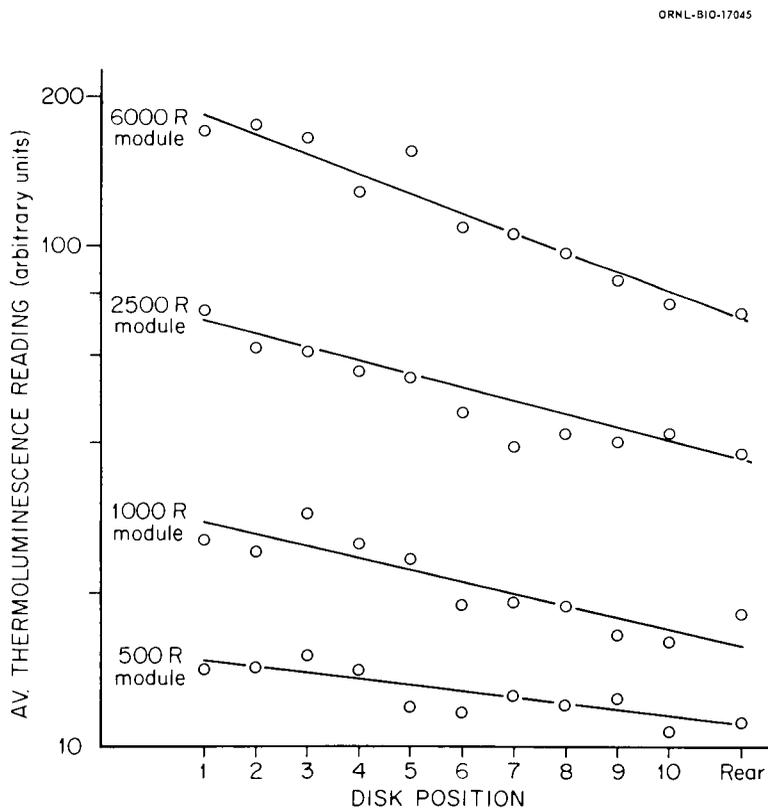


Fig. 6. Average Dosimeter Readings Plotted Against Module Position in Ames Dosimetry Exercise.

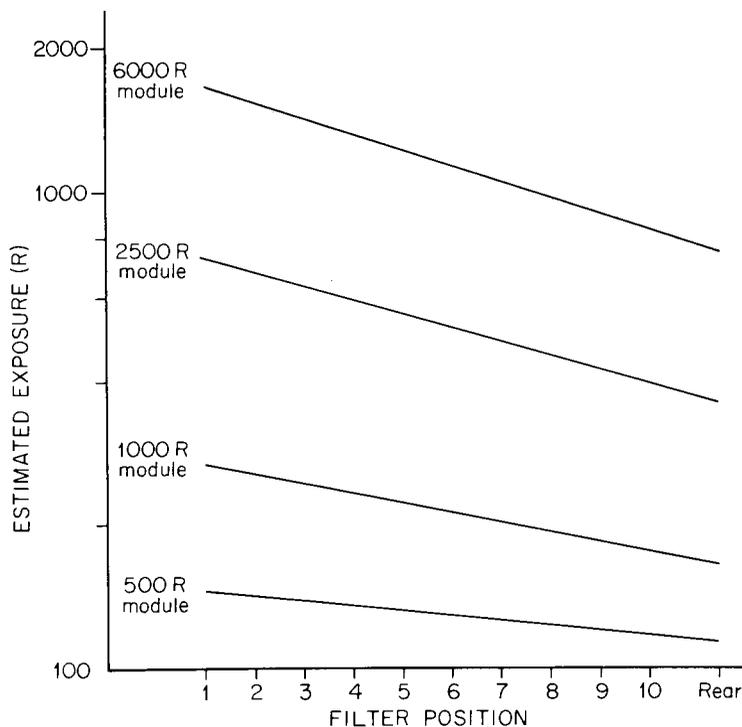


Fig. 7. Estimated Exposures to the Filter Positions in the Modules in the Ames Dosimetry Exercise.

thermoluminescence. This will be useful for comparison with data obtained by the Ames dosimetry group and could serve as an approximate correction factor for abstracting the conidial exposures from the exposures to lithium powder samples at the edges of each housing.

VI. INSTRUCTIONS FOR PACKAGE ASSEMBLY

A. Standing Instructions

A set of instructions for carrying out the flight preparations and the subsequent experimentation was required both as a guide for the Neurospora group and also to provide the means by which an objective observer could evaluate the preparations for any given test or flight situation. The procedure adopted at first was to write out in some detail all the operations which are carried out prior to and after a test or flight. This set of instructions is included in Appendix C.

B. Changes in Procedure

After this set of instructions was written, there were changes (listed below) in certain procedures, terminology, and materials resulting from experience with the flight-type hardware in tests at General Electric Company in Philadelphia.

(1) Appropriate terms (housing, module, and assembly) are used to replace the term "package" in accordance with the terminology of General Electric Company's manual (Biosatellite Program Document Number 65SD460). (2) It was decided that the housings would remain at the test or launch site prior to test or flight and that cleaning and preconditioning of the housings would be carried out there. Modules would be cleaned and loaded at ORNL. (3) Two relatively nontoxic detergents are specified ("7X" and "Haemo-sol"), either of which may be used for washing housings and/or modules. (4) Lithium fluoride-Teflon disk dosimetry is used to replace glass rod dosimetry. (5) Modules must be autoclaved in the assembled condition in paper bags with the screws torqued loosely. (6) Each housing which may be used for flight must be preconditioned (washed and autoclaved three times) and then tested in a storage test for absence of toxic effects before it is considered flight-ready. (7) Conidia are now strained through four separate thick premoistened cotton pads and washed thoroughly with water on a Millipore filter before being resuspended and subjected to conidial counts. (8) Dilution series are now carried out in steps of 1/10 dilution each (1 ml of suspension added to 9 ml of water for each step), and plating is done with 100 ml of medium per large petri dish (150 × 25 mm). (9) The retainer rings are now moistened before they are loaded so that the filters and conidia will not be dried out. (10) A half Dispo-plug in cap now replaces the circular sponge provided. (11) Torque specified for the module screws by General Electric Company was 20 in.-oz; experience with flight-type hardware indicates that this is too high, and the figure now being used is 8 in.-oz. (12) For tests at General Electric Company and at Cape Kennedy, the modules are removed from the housings under sterile conditions after each test, and the conidia are transported in modules to ORNL for analysis. Present plans call for the transportation of flight modules *in their housings* at ice-water temperature from the flight recovery area in Hawaii to ORNL for analysis. (13) Air flow setting for jugs has been changed from 0.04 to 0.033 arbitrary units on the standard air flowmeter.

C. Field Test Instructions

From the first set of instructions (Appendix C), certain portions have been selected which serve as good indicators of progress in the procedure and which are most concerned with handling of the flight hardware in preparation for a flight or test. These have been converted into a checklist (Appendix D) which is used by the inspector during test or flight preparations. The Field Test Instructions have been used in tests at Cape Kennedy and at General Electric Company and, with a few alterations since then, appear to provide a satisfactory guide for the use of the inspector at the test or launch site.

VII. STORAGE AND ANOXIA

A. General

Data concerning mutation rates, survivals, and radiation sensitivity from experiments in the laboratory have been applied to conidia in the flight and control vehicles. However, it is obvious that whereas most of the laboratory experiments were performed on well-aerated suspensions of conidia or conidia on the surface of Millipore filters in a large volume of air, the flight conditions would provide for a more limited supply of air. It was thought desirable to house the *Neurospora* conidia in closed packages, which would provide protection against toxic or mutagenic materials which might be present in the vehicle. However, it was felt that if the limited air supply seemed deleterious to the conidia, then perhaps some small change in assembly design could be executed to permit entry of air from the spacecraft through a sterility barrier. For this reason, studies on the effects of anoxia were initiated.

B. Survival in Air and in Nitrogen

In order to test whether artificially induced anoxia has a deleterious effect upon conidia, conidia in suspension were enclosed in modified gas washing bottles and bubbled at the beginning of the experiment and at intervals during the storage interval (at 4, 18, or 35°C) with air or with nitrogen. Conidia were removed from the bottles after 3, 6, 9, and 22 days without exposing the anoxic conidia to air. Survival of heterokaryotic conidia was assayed in each sample. The results, presented in Table 1, indicate that survival is best at the lowest temperature, but that at each temperature, heterokaryotic survival is lower under anoxic conditions. The 18°C data (approximating flight conditions) suggest that survival is satisfactory through nine days of incubation, provided there is an adequate air supply.

Table 1. Percentage Survival of Heterokaryotic Conidia After Incubation for Various Times and Under Different Atmospheric and Temperature Conditions

Temperature (°C)	Atmosphere	Time (days)				
		0	3	6	9	22
0 to 4	Air	(100)	96	97	90	63
18	Air	(100)	92	83	76	49
35	Air	(100)	85	40	28	1
0 to 4	Nitrogen	(100)	62	50	51	30
18	Nitrogen	(100)	9	10	7	2
35	Nitrogen	(100)	5	2	0.03	0.0001

C. Sensitivity to X Irradiation Under Anoxic Conditions

Mixed heterokaryotic and homokaryotic conidia were suspended and bubbled in small plastic bottles for 30 min with high-purity nitrogen, then irradiated, with continued nitrogen bubbling, with exposures of 5000, 10,000, 20,000, or 40,000 r of x rays (250 kvp, 30 ma, 3 mm aluminum extrinsic filtration).

Survival of heterokaryotic and homokaryotic conidia and of both types combined was assayed and compared with that for conidia subjected to the same exposures, but bubbled with air. The data are plotted in Fig. 8. It was shown that artificially induced anoxia alters the radiation sensitivity of *Neurospora*, protecting both heterokaryotic and homokaryotic conidia from killing with a dose-reduction factor of about 2. Similar data were obtained with mixed conidia on Millipore filters irradiated in a chamber flushed with nitrogen or with air. Kølmark [*Mutation Res.* 2, 222-28 (1965)] has previously shown this effect of anoxia against x-ray-induced reverse mutation.

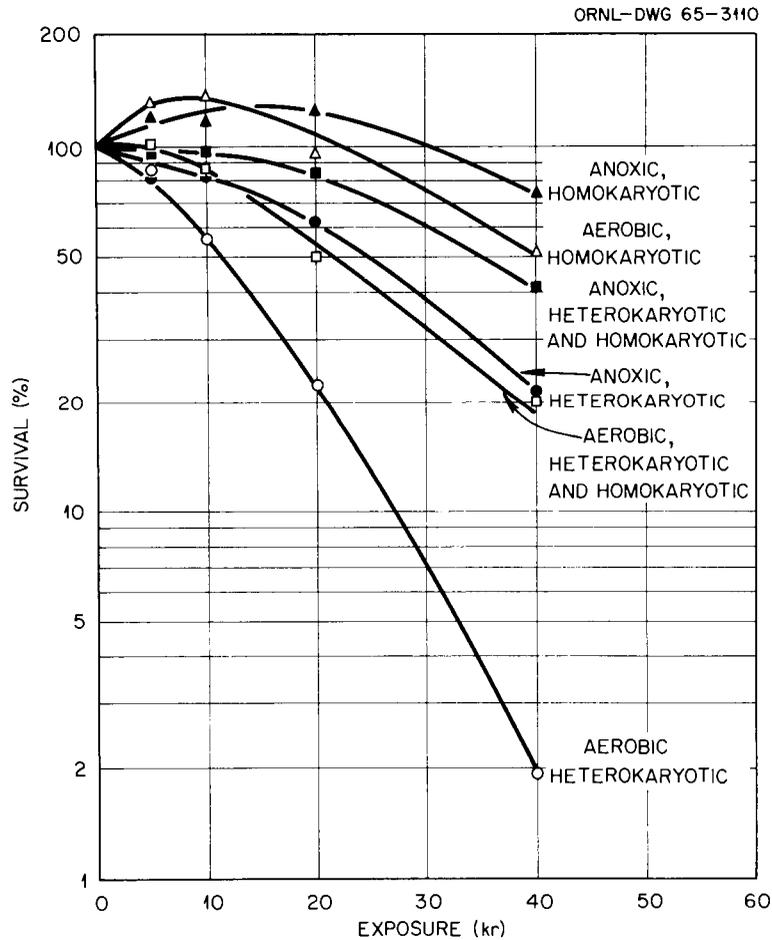


Fig. 8. Dose-Survival Curves for Conidia X Irradiated Under Aerobic and Anoxic Conditions.

D. Storage, Oxygen Content, and Conidia Survival

In preliminary experiments, gas sampling was attempted unsuccessfully from prototype housings in which conidia had been stored. Accordingly, simulated housings were fabricated from Pyrex and fitted with gas sampling ports, and conidia prepared and packaged in modules in the usual way were incubated at room temperature in these vessels. The air was sampled from one vessel each day and analyzed by means of mass spectrometry, and a conidial sample was plated from the vessel to assay heterokaryotic and total conidial survivals. The mass spectrometry results indicate that oxygen content dropped from 21% to about 16 to 17% and carbon dioxide increased from about 0.003 to about 3 to 5% during the first day of storage, and that no further change occurred over a seven-day period. The survival data for conidia stored under these conditions are plotted in Fig. 9 and show that although the homokaryotic conidia show a total decrease in survival (attributed to vitaminless death) over an 11-day period, the survival of heterokaryotic conidia at the end of this interval is still quite satisfactory (about 87%). The

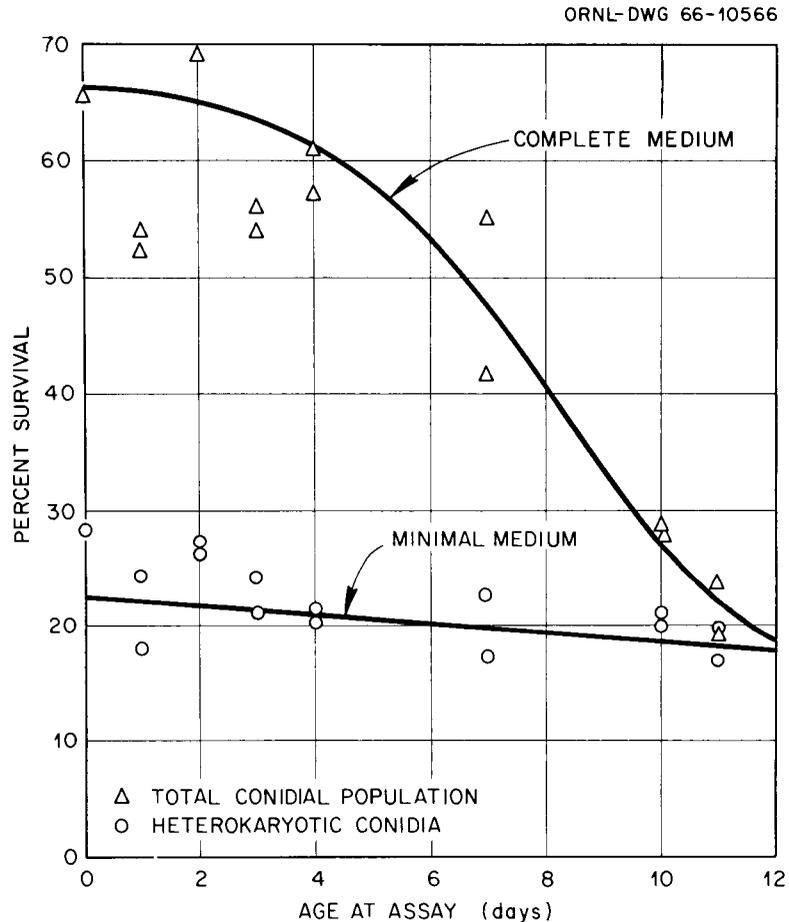


Fig. 9. Survival of Conidia of a Genetically Marked Two-Component Heterokaryon of *Neurospora crassa* in Simulated Three-Day Biosatellite Hardware at Room Temperature. (△ = total conidial population, ○ = heterokaryotic conidia.)

results indicate that anaerobiosis should not be a problem during the three-day flight and that survival of the heterokaryotic conidia which will be used in the assay system should not be affected by postflight storage during the interval anticipated.

E. Storage Time After Irradiation

Although the heterokaryotic survival of unirradiated conidia seems to remain high during storage, it was thought conceivable that storage might have an effect upon conidial sensitivity to ionizing radiation or upon mutant expression after irradiation. It was decided to look at the latter possibility in an experiment in which conidia were irradiated with 5000 r of x rays (250 kvp, 30 ma, 3 mm aluminum filtration) and then stored at room temperature in modules and a module-carrying box. Individual filters of conidia were sampled immediately after irradiation and then at intervals of two to three days, and conidia were assayed for mutation in the *ad-3* region and for heterokaryotic survival. The results, presented in Table 2, suggest an increase in mutation rate during the first two days and then again after about nine days. The increase in mutation rate during the first two to three days of postirradiation storage was also found in the previously reported dose-rate experiment (Sect. III-B) and is attributed to cytoplasmic repair, which improves the morphology of the colonies and results in better pigmentation and more complete recovery of purple (*ad-3*) mutants. The second increase in mutation frequencies after nine days of postirradiation storage seems to be correlated with a decrease in heterokaryotic survival and so may perhaps be attributed to differential survival of mutant and nonmutant conidia. In any case, the schedule for return of biological material from the biosatellite flight is such that jug inoculations should occur not less than two days after the end of irradiation and not more than nine days after the beginning of the irradiation, during which interval the relation between mutant frequency and radiation exposure seems rather constant.

Table 2. Forward-Mutation Frequencies from Individual Jugs Inoculated with Conidia Stored for Various Intervals at Room Temperature After Irradiation with 5000 r of X Rays

Postirradiation Storage Interval (days)	Mean Percent Heterokaryotic Survival	Mutants per 10 ⁶ Survivors
0	14.9	94.6; 89.8; 90.6
2	12.9	128.6; 114.6; 141.8
4	14.1	126.9; 122.1; 118.8
7	13.6	97.0; 107.3; 117.4
9	13.7	119.4; 103.2; 144.3
11	10.0	188.2; 154.7; 163.5
14	11.6	128.2
16	8.6	175.4
18	11.5	166.7; 142.5; 138.4
21	10.1	140.0; 193.8; 147.9

VIII. BIOCOMPATIBILITY TESTING

A. Introduction

We were informed that after the General Electric Company had completed component testing on the hardware the experimenters would be expected to participate in numerous tests on the systems level. On the basis of information provided in the experimenters' meeting in February 1966 at Philadelphia, the schedule of tests and experiments and of hardware requirements in Table 3 was prepared and used as a basis for planning a biological testing program. (It should be noted that the dates given were the earliest possible; it was anticipated that slips in component or other testing could cause subsequent changes in the schedule. It will also be apparent that the number and types of tests were subsequently altered; for example, biology was not used in the acoustic noise and impact shock qualification tests, the gantry exercises at Cape Kennedy

Table 3. Neurospora Experiment - (P-1037)

March 19, 1966

Test or Experiment	Week	Location	Type	Hardware	
				Number Required ^a	
				Housings	Modules
Anoxia tests	13-15	ORNL	Prototype		
Vibration qualification	17-19	G.E.	Qualification	5	10
Prime vehicle acceptance	18	G.E.	Flight	10	15
			Qualification	5	10
Acoustic noise qualification	20-21	G.E.	Qualification	5	10
Backup vehicle acceptance	21	G.E.	Flight	10	15
			Qualification	5	10
Thermal/vacuum qualification	22-23	G.E.	Qualification	5	10
Ames radiation group tests	23-24	Ames	Qualification	5	10
Impact shock qualification	25-26	G.E.	Qualification	5	10
Long-term biocompatibility	24-27	ORNL	Component qualification	1	1
Separation shock qualification	28	G.E.	Qualification	5	10
Biological check of backup hardware I		Cape	Flight	5	10
Biological check of backup hardware II		Cape	Flight	5	10
Biological check of prime hardware I		Cape	Flight	5	10
Biological check of prime hardware II		Cape	Flight	5	10
Biological check of control hardware I		Cape	Qualification	5	5
Biological check of control hardware II		Cape	Qualification	5	5
Flight (prime hardware)		Cape	Flight	10	10(per 2 days)
			Qualification	5	5(per 2 days)
Flight (backup hardware)		Cape	Flight	10	10(per 2 days)
			Qualification	5	5(per 2 days)
Postflight control		Ames	Flight	5	10

^aThese hardware requirements did not take into account the fact that six flight assemblies (including one spare) should be loaded for each test and for each flight loading sequence.

were reduced in number, and the thermal/vacuum qualification test was performed three times.) In the qualification tests, a set of flight-type hardware (not the flight items) was to be tested for structural integrity and function during and/or after subjecting the set to environmental stresses at 1.5 times the intensity anticipated for the flight. In the acceptance tests, the actual flight hardware is used and subjected to stresses at estimated flight intensities.

The primary reason for using the biology in these tests was to provide assurance to all investigators that the hardware would actually be capable of supporting each experiment. It was also possible in these tests to gain some experience in administering the deployment of personnel for a rather complex traveling schedule, to provide experience for technical personnel who would be involved in travel in connection with the flight in handling the hardware with biological samples outside of the laboratory, to test and modify where necessary the Standing Instructions for handling the hardware, and to develop, with the other investigators and the General Electric assembling personnel, the necessary teamwork to assemble and insert the payload according to schedule.

B. Biological Testing Procedure for Philadelphia Tests

The testing program was used to provide additional answers (see Sect. VII) to the question of whether storage of conidia in the biosatellite modules and housings under particular test conditions affects (1) survival of unirradiated conidia, (2) sensitivity to nuclear inactivation by x rays, (3) sensitivity to mutation induction by x rays, (4) survival of conidia irradiated before storage, and (5) mutation expression in populations of conidia irradiated before storage. The procedure planned for tests without radiation sources was to load two filters into each module with conidia which had been exposed to 5000 r of x rays at ORNL before the test storage period and eight filters with unirradiated conidia, two of which were similarly irradiated after the test storage period. (In practice, samples were irradiated *after* storage for very few of the experiments.) Survival of heterokaryotic conidia and of both classes of homokaryotic conidia was to be assayed by plating in selective media for all tests. Forward-mutation frequencies were to be obtained by jug experiments for some of the tests. Biological data for the Neurospora portion of these tests are presented in Appendix E. From the platings, only the data from minimal medium, which supports the growth of heterokaryotic conidia, will be presented, although similar data for supplemented media exist. The reason for this restriction is that the medium in the jugs also selects for heterokaryotic colonies, and so it is their survival in which we are most interested. Furthermore, the loss of survival of heterokaryotic conidia seems to be a one-hit phenomenon with x rays and gamma radiation, and so this is the kind of data best suited for use in assaying cellular inactivation.

It was observed early in the testing program that the survivals from vibration tests as well as from the Ames biocompatibility test seemed to be lower than those from thermal/vacuum tests. This difference was attributed to toxic products released from the plastic housings during

autoclaving. The recommended solution for this bioincompatibility problem was to autoclave and wash each housing at least three times to release and remove possible toxic products before using the housing in flight. This treatment is being applied to housings and its effectiveness evaluated in storage tests.

C. Ames Biocompatibility Tests

The tests at Philadelphia were all carried out without a radiation source in the source holder. However, at Ames Research Center, three tests were conducted with radiation sources. These are reviewed briefly in Sect. III-C; however, it would be desirable to describe the last of these, which was carried out with flight-type hardware, since the data and procedures are similar to those expected from the flight. This test was conducted to assure the investigators that the flight-type hardware will support a radiation experiment with a live radiation source, to provide the experimenters with control data obtained with the flight-type hardware, to provide further practice in loading and assembling the flight-type hardware on schedule, and to test again for an effect of a vibration and centrifugation profile simulating flight.

Dosimeters were exposed by Dr. Hewitt to ^{85}Sr gamma radiation, and total exposures measured by reference to a thimble dosimeter appropriately calibrated for that type of radiation. The calibration data and curve are presented in Table 4 and Fig. 10. The thermoluminescence readings from dosimeters adjacent to the filter No. 1 conidia can be used to abstract from the calibration table the exposure received by the conidia. The radiation exposure estimates are presented in Table 5, and the mutation frequencies and survival data for the filter No. 1 conidia are listed in Table 6 and indicated in Fig. 11. Although there is no obvious relationship between survival and radiation exposure, the mutation frequencies seem to increase with increasing exposure. The means are included in Fig. 1, and seem to fit well with the data for x rays and other gamma radiation experiments. The mutation frequencies for vibrated conidia are plotted lower than for unvibrated conidia (though the differences are probably not significant), so that there is surely not an enhancement of mutant induction by the vibration and centrifugation profiles used.

Table 4. Thermoluminescence Readings for Lithium Fluoride Disk Dosimeters Which Received Known Exposures of ^{85}Sr Gamma Radiation (Calibration Data)

Nominal Exposure (r)	Thermoluminescence Readings	
	Individual Disks	Mean
6180	1060	1224
	1330	
	1282	
3300	441.0	480.4
	408.3	
	591.9	
1525	140.6	162.9
	167.3	
	180.8	
850	91.8	81.9
	76.2	
	77.8	
412	40.3	40.2
	40.0	
207	27.5	21.6
	15.6	
106	11.0	9.9
	8.8	

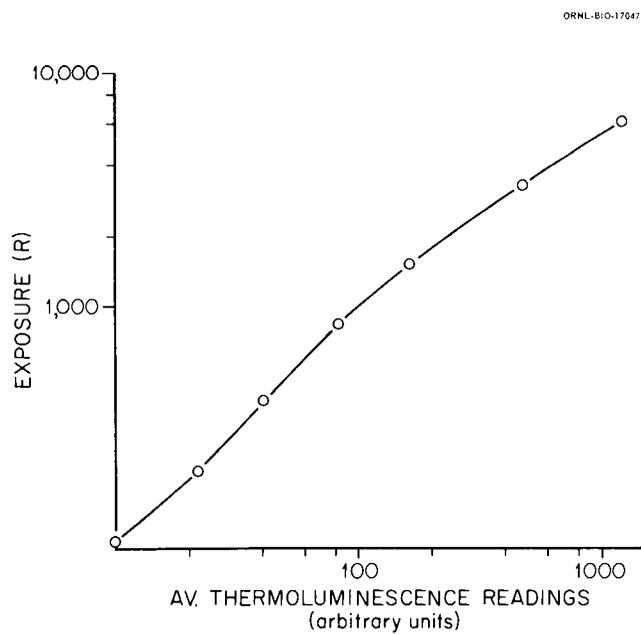


Fig. 10. Calibration Curve for ^{85}Sr Exposure for Ames Biocompatibility Test.

Table 5. Thermoluminescence Readings from Dosimeters Exposed with Biology and Estimated Dose (from Calibration Curve)

Nominal Exposure to Package	Thermoluminescence Readings, Filter No. 1		Estimated Exposure to Filter No. 1 (r)
	Individual Filters	Mean	
Vibrated, 6000 r	719.8	710.5	4300
	624.0		
	787.8		
Unvibrated, 6000 r	782.0	841.4	4800
	837.2		
	905.0		
Vibrated, 2500 r	244.6	259.8	2150
	287.0		
	247.9		
Unvibrated, 2500 r	297.6	284.4	2300
	257.7		
	297.8		
Vibrated, 1000 r	67.6	77.8	880
	87.7		
	78.0		
Unvibrated, 1000 r	84.8	72.7	750
	71.4		
	61.8		

Table 6. Ames Biocompatibility Test No. 201 Special Flight-Type Hardware

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Heterokaryotic Survival and Mutation Data from Individual Jugs

Test Position (Filter No. 1)		Mutation Frequency			Heterokaryotic Survival		
Treatment	Estimated Exposure (r)	Individual Jugs	Mean	95% Confidence Interval	Individual Jugs	Mean	Percent of Original Heterokaryotic Fraction (0.2046)
		$\times 10^{-6}$	$\times 10^{-6}$	$\times 10^{-6}$			
Vibrated, 6000 r	4300	65.3	66.6	47.3-85.9	0.1169	0.1305	63.78
		73.3			0.1410		
		50.6			0.1422		
		67.9			0.1258		
		75.8			0.1265		
Unvibrated, 6000 r	4800	76.6	95.4	80.5-110.3	0.0973	0.0890	43.50
		85.6			0.0858		
		104.7			0.0899		
		95.6			0.0831		
		114.3					
Vibrated, 2500 r	2150	25.5	24.8	13.9-35.7	0.1205	0.1275	62.32
		32.6			0.1203		
		17.2			0.1294		
		23.1			0.1407		
		25.6			0.1267		
Unvibrated, 2500 r	2300	47.3	39.3	19.6-59.0	0.0737	0.0677	33.09
		48.1			0.0648		
		34.1			0.0593		
		27.7			0.0729		
Vibrated, 1000 r	880	12.6	11.3	8.2-14.4	0.1175	0.1321	64.57
		12.6			0.1176		
		12.1			0.1361		
		9.1			0.1617		
		10.3			0.1274		
Unvibrated, 1000 r	750	8.4	10.0	5.8-14.2	0.0981	0.0990	48.39
		8.4			0.0782		
		12.7			0.1039		
		11.9			0.0969		
		8.4			0.1179		
Vibrated, 0 r	0	0	0.5		0.1000	0.0920	44.97
		0			0.0914		
		0			0.0869		
		1.9			0.0895		
Unvibrated, 0 r	0	0	0.6		0.1329	0.1414	69.11
		0			0.1487		
		2.4			0.1415		
		0			0.1425		

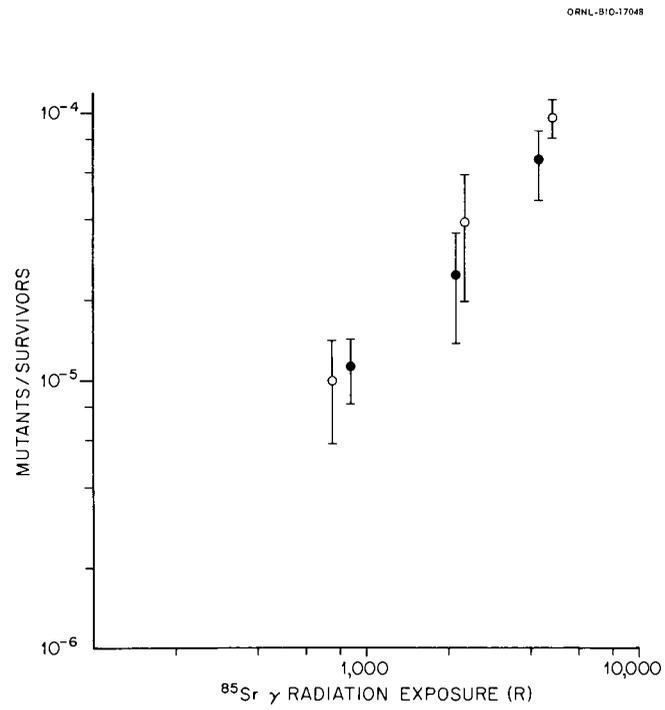


Fig. 11. Mutation Frequency Plotted Against ^{85}Sr Gamma Radiation Exposure in Ames Biocompatibility Experiment. Intervals are 95% confidence limits ($\text{mean} \pm 1.96 \times \text{sample standard error}$). ● vibrated; ○ unvibrated.



APPENDIX A



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INDUCTION KINETICS AND GENETIC ANALYSIS OF X-RAY-INDUCED
MUTATIONS IN THE AD-3 REGION OF NEUROSPORA CRASSA*,**

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Forward-mutation experiments with a genetically marked balanced heterokaryon of *Neurospora* have shown that two different classes of mutations are induced in the ad-3 region by X-irradiation.¹ The first class consists of reparable mutants (ad-3^R) that will grow as homokaryons on adenine-supplemented medium, and the second consists of irreparable mutants (ad-3^{IR}) that will not grow as homokaryons either on adenine-supplemented or complete medium.² Genetic analyses by means of homology tests^{2,3} indicate that the ad-3^R mutants have only the ad-3A or ad-3B locus inactivated, whereas in the ad-3^{IR} mutants the inactivation covers other loci in the immediately adjacent regions. The most reasonable explanation for these data is that the two classes of mutants result from different types of genetic alteration. These differences can be explained if each ad-3^R mutation is a point mutation resulting from a single alteration within the ad-3A or ad-3B locus, and each ad-3^{IR} mutation is produced by a chromosome deletion resulting from two alterations — with one or both outside of the ad-3A and ad-3B loci. If this is true, then the difference between ad-3^R and ad-3^{IR} mutations should be reflected in their induction kinetics.

The development of a direct forward-mutation method for use with wild-type strains of *Neurospora*⁴ has made it possible to obtain precise forward-mutation frequencies for the induction of ad-3 mutants after mutagenic treatment. In the present experiment, the method has been used to study the kinetics of induction of forward mutations in the ad-3 region after exposure to X rays. The experiment was designed to investigate

these genetic effects over the widest possible range of survival levels. Data derived from genetic analysis of random samples of mutants induced by 1, 2, 5, 10, 20, and 40 kr exposures demonstrate a difference in the induction kinetics of ad-3^R mutants and ad-3^{IR} mutants and describe the dose dependence of the spectrum of X-ray-induced mutation in the ad-3 region.

Materials and Methods.—Strains: The strain numbers and genetic markers in the two-component heterokaryon (referred to as a dikaryon) are given in Table 1. The hist-2 marker is located in the right arm of linkage group I (LG IR) near the centromere and shows 0.154% crossing over.⁵ The ad-3 loci are about 2.0 map units to the right of hist-2, and the nic-2 locus is about 5.0 map units to the right of hist-2.⁶ Conidia in the dikaryotic culture are of three different types, namely, (1) homokaryotic for component I; (2) homokaryotic for component II; and (3) heterokaryotic, containing at least one nucleus of each type. Only the heterokaryotic conidia grow on minimal medium, and those heterokaryotic conidia containing mutations in the ad-3 region in component II can be recovered by supplementing this medium with adenine. The average nuclear number per heterokaryotic conidium has been minimized by growing the culture on minimal medium⁷ and by filtering the conidial suspension through thick cotton pads.

The present dikaryon differs from the one used in earlier experiments¹ by the presence of an ad-2 marker in linkage group III (LG III) in component I. The ad-2 block precedes the ad-3 block (or blocks) in purine biosynthesis, so that the presence of this marker prevents the formation and accumulation of purple pigment in heterokaryotic colonies with a skewed nuclear ratio

(T-1)

favoring component I. The presence of the ad-2 marker insures that purple colonies develop only from those heterokaryotic conidia in which the ad-3A and/or the ad-3B locus in component II has been inactivated.

Isolation of mutants: The general procedure and media for forward-mutation experiments with homokaryotic wild-type strains of *Neurospora* have been described previously.⁴ Modifications in procedure for experiments with a dikaryon are as follows. To minimize the recovery of spontaneous ad-3 mutants, single colony isolates (from conidia of the dikaryon plated at 35°C in minimal medium) were made, and these were grown on minimal medium for 2 days at 35°C and then 5 to 8 days at 24°C. Cotton-filtered suspensions of conidia in water from several of these cultures were adjusted to a concentration of 10^7 per ml by means of hemocytometer counts, and aliquots were irradiated (in Erlenmeyer flasks stirred with a magnetic stirrer) at ice-water temperature with a General Electric Maxitron 250 X-ray source (operated at 250 kv, 30 mA, with 3 mm Al filter) at an exposure rate of 1000 r/min. After the desired exposures had been given, aliquots of suspension containing about 10^6 surviving heterokaryotic conidia were each inoculated into 10 liters of medium containing 12.5 mg/l adenine sulfate, 250 mg/l L-arginine HCl, 10 mg/l nicotinamide, 1.5% sorbose, and 0.1% sucrose in 12-liter Florence flasks and incubated at 30°C for 6 days with aeration in the dark. During this time, each surviving conidium grows into a colony about 2 mm in diameter. The contents of each flask were determined volumetrically and examined for the presence of purple colonies in thin layers in white photographic

developing trays. The total number of purple ad-3 mutant colonies per flask was determined, and each one was isolated and subcultured; the total number of unpigmented colonies was estimated from counts on three samples of 2 or 6 ml taken from each flask, in addition to measurements of the total volume. Heterokaryotic survival was estimated from counts of unpigmented colonies and hemocytometer counts of the original conidial suspension. Estimates of forward-mutation frequency are based on the colony counts of pigmented and unpigmented colonies from each of the incubation flasks and are expressed as ad-3 mutants (purple colonies) per 10^6 heterokaryotic survivors (total colonies).

Genetic analysis: Conidia from random samples of subcultures of the purple colonies obtained from each exposure were plated, and single-colony isolates that were heterokaryotic (with regard to the two different components of the dikaryon) and adenine-requiring were obtained for each of the purple colonies originally isolated. These single-colony isolates were then analyzed as indicated below to determine (1) genotype (whether ad-3A, ad-3B, nic-2, or any combination of these) and (2) whether the ad-3 mutation was reparable or irreparable on adenine-supplemented medium. Since histidine supplementation inhibits the general recovery of ad-3 mutations as purple colonies, ad-3 mutations affecting the hist-2 locus were not sought.

Genotype: An ad-3 dikaryon representing each initial colony was analyzed in heterokaryon tests with four different tester strains on minimal medium to determine which of the known loci in the vicinity of the ad-3 region had been inactivated. The genotype and numbers of each tester

strain are as follows: (1) ad-3A — 1-112-13; (2) ad-3B — 1-112-2; (3) hist-2 nic-2 al-2 — 74-OR33-3A; and (4) ad-2 inos — 74-OR60-44A. Tester 4 serves as a control, and the heterokaryon test responses with tester strains 1, 2, and 3 indicate whether the ad-3A locus, the ad-3B locus, and/or the nic-2 locus, respectively, is inactivated. Each of the four testers bears at least one biochemical marker which is also present in component I; therefore, any growth in minimal medium must reflect complementation between the tester and component II of the dikaryon.

Tests to distinguish between $ad-3^R$ and $ad-3^{IR}$ mutations: Dikaryon test. Conidia (typically 500 to 2000) from each dikaryon were plated in medium supplemented with 100 mg/l adenine sulfate and 2 mg/l calcium pantothenate and incubated at 35°C for 60 to 72 hours. Reparability of the ad-3 mutation in component II is indicated by the presence of cot colonies, which are also homokaryotic for al-2 and pan-2. In the dikaryon test it is not possible to distinguish ad-3^{IR} mutations from those that are ad-3^R + RL, where RL designates recessive lethal damage elsewhere in the genome.² Either type of mutation results in homokaryotic lethality of component II and the absence of cot colonies in the dikaryon test.

Trikaryon tests. The ad-3^{IR} and ad-3^R + RL mutations can be distinguished by a trikaryon test employing the dikaryotic tester strain 12-1-18, which was obtained by X-irradiation of the dikaryon described in Table 1 and which carries a deletion for ad-3A, ad-3B, and nic-2

in component II. The dikaryon to be tested is combined with dikaryon 12-1-18, and conidia from the resulting trikaryon (not a tetrakaryon, since component I in each dikaryon is identical) are plated to determine whether dikaryotic cot colonies are formed. If cot colonies are present in the trikaryon test with tester 12-1-18 but absent from the dikaryon test, then component II bears irreparable damage outside of the region covered by the 12-1-18 deletion. The absence of cot colonies in both the dikaryon test and the trikaryon test indicates that the irreparable damage is within the region covered by the 12-1-18 deletion, and the strain being tested is most probably a true ad-3^{IR} mutation.

Dikaryons showing negative results in the dikaryon test and the trikaryon test with tester 12-1-18 were subjected to additional trikaryon tests with an ad-3A^{IR} tester strain (12-7-215) and an ad-3B^{IR} tester strain (12-5-182), each of which has been shown in extensive homology tests (F. J. de Serres, unpublished) to have irreparable damage closely associated with the inactivated ad-3 locus. Such tests permit a distinction between ad-3^{IR} mutations and ad-3^R + RL mutations, where RL designates a separate site of recessive lethal damage closely linked with the ad-3A and ad-3B loci. The use of trikaryon tests is discussed more fully elsewhere.²

Results.—Survival of heterokaryotic fraction of conidia: The survival of heterokaryotic conidia after various X-ray exposures from four separate experiments, as well as averages from the four experiments combined, are plotted in Figure 1. The decrease in heterokaryotic survival with increasing (F-1) dose appears to be exponential. Atwood and Mukai⁸ have previously shown that after X-irradiation the survival of the total conidial population

of a heterokaryon (both heterokaryotic and homokaryotic conidia) exhibits multi-hit kinetics, while the survival of the heterokaryotic fraction is exponential or one-hit in nature. The more rapid inactivation of the heterokaryotic conidia is attributed to nuclear inactivation that results in the rapid conversion of binucleate heterokaryotic conidia into uninucleate (and homokaryotic) conidia which can no longer grow on minimal medium.

Relation between ad-3 mutant frequency and dose: In Table 2 and Figure 2 the ad-3 mutant frequencies at 1, 2, 5, 10, 20, and 40 kr exposures are presented. The mutant frequencies increase in proportion to the 1.36 power of the dose. This exponent is significantly different from 1.0 ($P < 0.001$).

T-2

F-2

Genetic characterization of ad-3 mutants from each dose: The results of the tests described in Materials and Methods for genotype and for reparability of a random sample of mutants from each X-ray dose are presented in Table 3. The numbers of mutants in each of the three classes thus defined (ad-3^R, ad-3^{IR}, and ad-3^R + RL) are included in Table 3. All mutants tested are also classified with regard to genotype as ad-3A mutants, ad-3B mutants, or mutants inactivated in two or more loci (ad-3A ad-3B, ad-3A ad-3B nic-2, or ad-3B nic-2, all of which are irreparable).

T-3

Estimates of the frequency (expressed as a function of survivors) of all reparable mutants (including both ad-3^R and ad-3^R + RL), of irreparable mutants, and of reparable mutants with irreparable damage elsewhere in the genome (ad-3^R + RL) may be obtained by multiplying the proportion of tested mutants which falls into each of the categories at any dose by the ad-3

mutant recovery at that dose. Such estimates are listed in Table 3 for ad-3^R, ad-3^{IR}, and ad-3^R + RL mutants, and in Figure 3 these estimates are plotted as a function of dose. Ad-3^R mutants increase in proportion to the dose, while ad-3^{IR} and ad-3^R + RL mutants increase as the square of the dose. In other words, ad-3 mutants, which increase as the 1.36 power of the dose, consist of two classes of mutants, namely, ad-3^R mutants, which exhibit single-hit kinetics, and ad-3^{IR} mutants, which exhibit two-hit kinetics. Some ad-3^R mutants have acquired a second independent alteration elsewhere in the genome which causes irreparable damage unrelated to the mutation affecting the ad-3 locus. Mutants of this type (ad-3^R + RL) also exhibit two-hit kinetics, as was expected. The actual estimates of the exponent b in the equation $y = ax^b$ (where y = mutant yield and x = X-ray exposure) for all ad-3^R mutants and for ad-3^{IR} mutants are 1.08 and 2.09, respectively; these estimates are not significantly different from 1.0 and 2.0 (P = 0.2-0.3 and P = 0.4-0.5, respectively). The point estimate of this parameter for ad-3^R + RL mutants is 1.84, which approximates 2.0; the difference is barely statistically significant (with P slightly less than 0.05), but the regression analysis of ad-3^R + RL mutants is based on only five dose-response points, each of which represents a much smaller number of mutants than the other analyses.

Discussion.—Two classes of mutants: Two different types of ad-3 mutants are recovered after X-irradiation. One type (ad-3^R) is the predominant type at low doses (and low mutant recovery frequencies), and

this type increases in proportion to the dose. Mutations of this type result from intragenic alterations. The second type of mutant (ad-3^{IR}) is almost negligible in its incidence at low doses, but this increases as the square of the dose, so that at 40 kr, where mutant recovery frequencies are high, about 2/3 of the mutants are ad-3^{IR}. The dose-squared kinetics suggest that each ad-3^{IR} mutant is derived from the cooperative effect of two alterations which are independent in their probabilities of occurrence.

The mode of origin of ad-3^R and ad-3^{IR} mutations: The genetic analysis shows that ad-3^R and ad-3^{IR} mutants have different modes of origin. One might have expected that ad-3^R mutations would be converted to ad-3^{IR} mutations by additional hits in one of the immediately adjacent genetic regions (thus causing interstitial deletions rather than point mutations). However, if this were true, the frequency of ad-3^R mutants should increase as some power of the dose less than 1.0. Neither the combined induction curve for all ad-3^R mutants (Fig. 3) nor the individual induction frequencies for ad-3A^R and ad-3B^R mutants show a power of the dose significantly different from 1.0, even at the highest doses. This must mean that each ad-3^{IR} mutation results from a pair of mutually independent events which are separately not detectable as ad-3^R mutations. These independent events are tentatively viewed as alterations or breaks on opposite sides of one or both of the ad-3 loci which interact to delete the genetic region between them.

Forward mutation rates in the ad-3 region: The yield y of ad-3 mutants at any X-ray dose x may be described by the equation $y = k + ax + bx^2$.

Here \underline{k} equals the spontaneous mutant frequency (negligible in this case); \underline{ax} equals the yield of $\underline{ad-3^R}$ mutants; and $\underline{bx^2}$ equals the yield of $\underline{ad-3^{IR}}$ mutants. Since \underline{ax} and $\underline{bx^2}$ have been determined experimentally for each dose in the present paper, one may obtain values for \underline{a} and for \underline{b} by fitting the data derived from the genetic analysis with \underline{ax} and $\underline{bx^2}$, respectively. The term \underline{a} then represents the mutation rate for $\underline{ad-3^R}$ mutants and is found to be $5.39 \times 10^{-9} \underline{ad-3^R} \text{ mutants} \cdot \text{r}^{-1} \cdot \text{survivors}^{-1}$. The rate is 1.75×10^{-9} for $\underline{ad-3A^R}$ mutants and 3.64×10^{-9} for $\underline{ad-3B^R}$ mutants. On the other hand, the term \underline{b} is a compound term and represents the product of two independent probabilities, namely the probability per unit dose that the first of the two hits will occur and cooperate to produce an $\underline{ad-3^{IR}}$ mutation, times the probability that the second hit will do so. It seems reasonable to assume that these two probabilities are equal, because the hist-2 - ad-3 and the ad-3 - nic-2 regions are approximately equal in length. Accordingly, one may use $\sqrt{\underline{b}}$ ($4.01 \times 10^{-7} \text{ hits} \cdot \text{r}^{-1} \cdot \text{survivors}^{-1}$) as an approximation for the frequency of the occurrence and recovery of each of the two types of hits. This is necessarily a minimal estimate for frequency of the occurrence of these types of hits, since some will probably occur in the ad-3 region, but their location will be such that neither the ad-3A nor the ad-3B locus is deleted. Furthermore, the deficiency⁹ of ad-3A ad-3B double mutants and of ad-3^{IR} mutants which have lost the nic-2 locus suggests that the majority of ad-3^{IR} mutants result from pairs of breaks which are close together. For these reasons, $4.01 \times 10^{-7} \text{ hits} \cdot \text{r}^{-1} \cdot \text{survivors}^{-1}$ (about 74 times the frequency of events leading to $\underline{ad-3^R}$ mutants) considerably

underestimates the incidence of hits which are capable of interacting in pairs to produce irreparable mutants.

Implication for the comparison of X-ray-induced mutations in haploid and diploid organisms: The finding that the spectrum of X-ray-induced mutations in the ad-3 region includes both intragenic and extragenic alterations makes it possible to reconcile the differences observed in the analysis of X-ray-induced mutations in haploid and diploid organisms. Whereas in *Neurospora* there is evidence that X rays cause intragenic alterations,^{6,10,11} the evidence from similar studies with maize shows that all X-ray-induced mutations have the characteristics of extragenic alterations.¹²⁻¹⁴ The problem of the nature of X-ray-induced mutations in *Drosophila* is one of long standing, with two distinct schools of thought that have only recently been reconciled (see discussions by Muller and Oster,¹⁵ Lefevre and Green,¹⁶ and Green¹⁷). Some of these workers found only stable X-ray-induced mutations while others found revertible X-ray-induced mutations as well.

Since X-ray-induced mutations in the ad-3 region are a mixture of one-hit and two-hit alterations, one would expect this to be generally true for other loci in other organisms as well. In general, high exposures are frequently used in forward-mutation experiments to provide a high mutant yield. However, high doses favor extragenic (two-hit) alterations, and in diploid organisms these would constitute the majority class of mutants recovered. However, such irreparable mutations are lethal in forward-mutation studies with haploid organisms, and so in typical experiments with haploid organisms only the intragenic (one-hit)

alterations are recovered and analyzed. Whereas the two-hit alterations leading to chromosome deletions should be stable in reversion tests, de Serres⁶ has found that 25% of a sample of X-ray-induced ad-3^R mutants were revertible spontaneously or after X-ray treatment. Thus in experiments involving high doses of X rays with diploid organisms, the revertible mutants might constitute a very small fraction of the total number, and a large number of mutants would be required to demonstrate revertibility of X-ray-induced mutants. Data for revertibility of X-ray-induced mutants would be comparable in haploid and diploid organisms only if low X-ray exposures were used in the forward-mutation experiments, so that the majority of mutants would be one-hit in origin. In summary, our evidence indicates that a series of X-ray-induced allelic mutants should be composed of both stable and revertible alleles, and the percentages of each class should be dependent upon the dose and upon the efficiency with which all types of induced mutants can be recovered.

The nature of the genetic alterations in X-ray-induced ad-3^R mutants:
 The ad-3^R mutants clearly behave differently from ad-3^{IR} mutants in homology tests (de Serres² and unpublished); in ad-3^{IR} mutants the genetic inactivation frequently includes regions outside of the ad-3A or ad-3B cistrons, while in ad-3^R mutants the damage is invariably restricted to either the ad-3A or the ad-3B cistron. Although little is yet known from reverse-mutation studies about the types of alterations involved in production of ad-3^R mutants at the molecular level, some inferences can be made from studies of the phenotypes of X-ray-induced mutants. It has been shown that X-ray-induced ad-3^R mutants are less

likely to exhibit leakiness and allelic complementation than are mutants induced by 2-aminopurine or nitrous acid (de Serres;¹⁸ de Serres, Brockman, Barnett, and Kølmark, in preparation). Both polarized and nonpolarized complementation patterns occur among X-ray-induced mutants, suggesting that X rays can induce both base-pair substitutions and base-pair insertions or deletions. The X-ray-induced mutations which have been shown in previous experiments (e.g., de Serres⁶) to revert (either spontaneously or after X-ray treatment) may tentatively be attributed to these types of X-ray-induced genetic alterations.

Summary.--

(1) An analysis of the induction of ad-3^R and ad-3^{IR} mutations in a balanced dikaryon of Neurospora crassa with X-irradiation shows that they have different induction kinetics. Ad-3^R mutants show single-hit kinetics, while ad-3^{IR} mutants show two-hit kinetics.

(2) The spectrum of X-ray-induced forward mutation in the ad-3 region is dose-dependent. Whereas at 1 kr 97% of the mutants are ad-3^R (and affect only the ad-3A locus or ad-3B locus), at 40 kr 56% are ad-3^{IR} and 26% inactivate both the ad-3A and ad-3B loci.

(3) Estimates of the forward-mutation rates for the two loci in the ad-3 region are 1.75×10^{-9} mutants $\cdot r^{-1} \cdot \text{survivors}^{-1}$ for ad-3A^R mutants and 3.64×10^{-9} mutants $\cdot r^{-1} \cdot \text{survivors}^{-1}$ for ad-3B^R mutants. A minimal estimate for the incidence of the hits that cooperate to give ad-3^{IR} mutants is 4.01×10^{-7} hits $\cdot r^{-1} \cdot \text{survivors}^{-1}$.

(4) The finding that the spectrum of X-ray-induced mutations in the ad-3 region includes both intragenic and extragenic alterations makes it

possible to reconcile the differences observed in previous analyses of X-ray-induced mutations in haploid and diploid organisms.

We wish to acknowledge gratefully the aid of Dr. Marvin Kastenbaum in the statistical analysis, and the technical assistance of Mrs. Arlee Teasley, Miss Anita Meley, and Mrs. Ida Ruth Miller.

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TABLE 1

GENETIC COMPOSITION OF EACH COMPONENT OF THE DIKARYON
USED IN THE FORWARD-MUTATION EXPERIMENT

Linkage group	Component I	Component II
	(74-OR60-29A)	(74-OR31-16A)
IR	<u>hist-2</u> <u>ad-3A</u> <u>ad-3B</u> <u>nic-2</u>	<u>a1-2</u>
III	<u>ad-2</u>	—
IV	—	<u>cot</u>
V	<u>inos</u>	—
VI	—	<u>pan-2</u>

TABLE 2

SURVIVAL OF HETEROKARYOTIC CONIDIA AND THE FORWARD-MUTATION FREQUENCIES OF MUTATIONS IN THE ad-3 REGION AFTER EXPOSURES TO 250 KV X-RAYS (FRACTIONS IN PARENTHESES ARE THE NUMBERS OF PURPLE MUTANT COLONIES DIVIDED BY THE TOTAL NUMBER OF BACKGROUND COLONIES)

Exposure (Kr)	Survival of hetero- karyotic conidia (%)	Mutation frequencies				
		Experiment number				
		12-5	12-6	12-7	12-10	Average*
0	100	0.2 (1/4,301,000)	0.3 (1/3,025,000)	0.7 (2/4,734,000)	0.4 (1/2,778,000)	0.3 (5/14,838,000)
1	93	6.2 (19/3,050,000)	4.1 (14/3,390,000)	4.1 (73/17,932,000)		4.3 (106/24,372,000)
2	86	15.8 (54/3,412,000)	6.5 (16/2,456,000)	11.0 (74/6,740,000)		11.4 (144/12,608,000)
5	59	40.9 (130/3,179,000)	27.4 (57/2,080,000)	49.1 (73/1,486,000)	33.8 (77/2,279,000)	37.3 (337/9,024,000)
10	53	122.8 (610/4,966,000)	77.6 (242/3,120,000)	85.5 (209/2,444,000)	74.0 (183/2,475,000)	95.7 (1244/13,005,000)
20	29				248.9 (1580/6,349,000)	248.9 (1580/6,349,000)
40	7				460.6 (3004/6,522,000)	460.6 (3004/6,522,000)

*Survival of heterokaryotic conidia on minimal medium for each dose is a weighted average percentage from data in the four experiments. Average mutation frequencies are obtained by dividing the total number of purple colonies from all experiments by total number of colonies examined.

TABLE 3

CLASSIFICATION OF ad-3 MUTANTS BY GENOTYPE AND REPARABILITY

Dose (kr)	Genotype												Incidence per 10 ⁶ survivors		
	<u>ad-3A</u>				<u>ad-3B</u>				<u>ad-3A</u> <u>ad-3B*</u>	<u>ad-3A</u> <u>ad-3B</u> <u>nic-2*</u>	<u>ad-3B</u> <u>nic-2*</u>	Total	<u>ad-3</u> ^{R†}	<u>ad-3</u> ^R + RL	<u>ad-3</u> ^{IR}
0	1	0	0	1	3	0	0	3	0	0	0	4	0.3	0	0
1	27	2	0	29	67	0	1	68	2	0	0	99	4.2	0.1	0.1
2	24	0	3	27	68	0	2	70	2	0	0	99	10.6	0	0.8
5	62	2	2	66	98	6	10	114	6	2	0	188	33.3	1.6	4.0
10	44	4	16	64	97	10	22	129	29	5	2	229	64.8	5.9	30.9
20	20	3	3	26	34	8	14	56	24	3	0	109	148.3	25.1	100.5
40	8	6	10	24	21	9	19	49	24	0	2	99	204.6	69.8	255.8

*Mutants in the three classes, ad-3A ad-3B, ad-3A ad-3B nic-2, and ad-3B nic-2, are all irreparable.

†Mutants scored as ad-3^R and mutants scored as ad-3^R + RL are included.

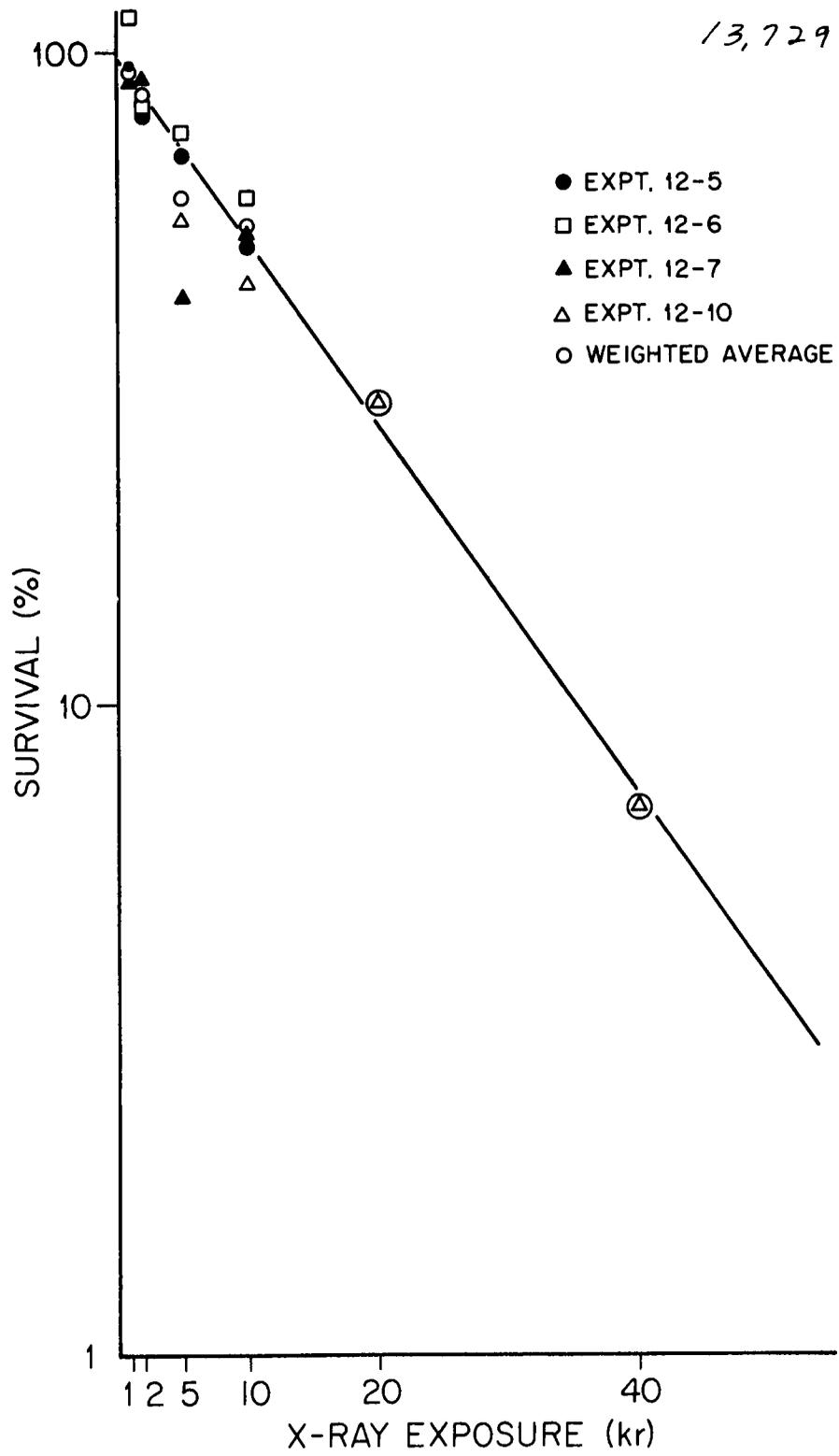


FIG. 1. — Survival of heterokaryotic conidia after various exposures to 250 kv X rays.

13,730

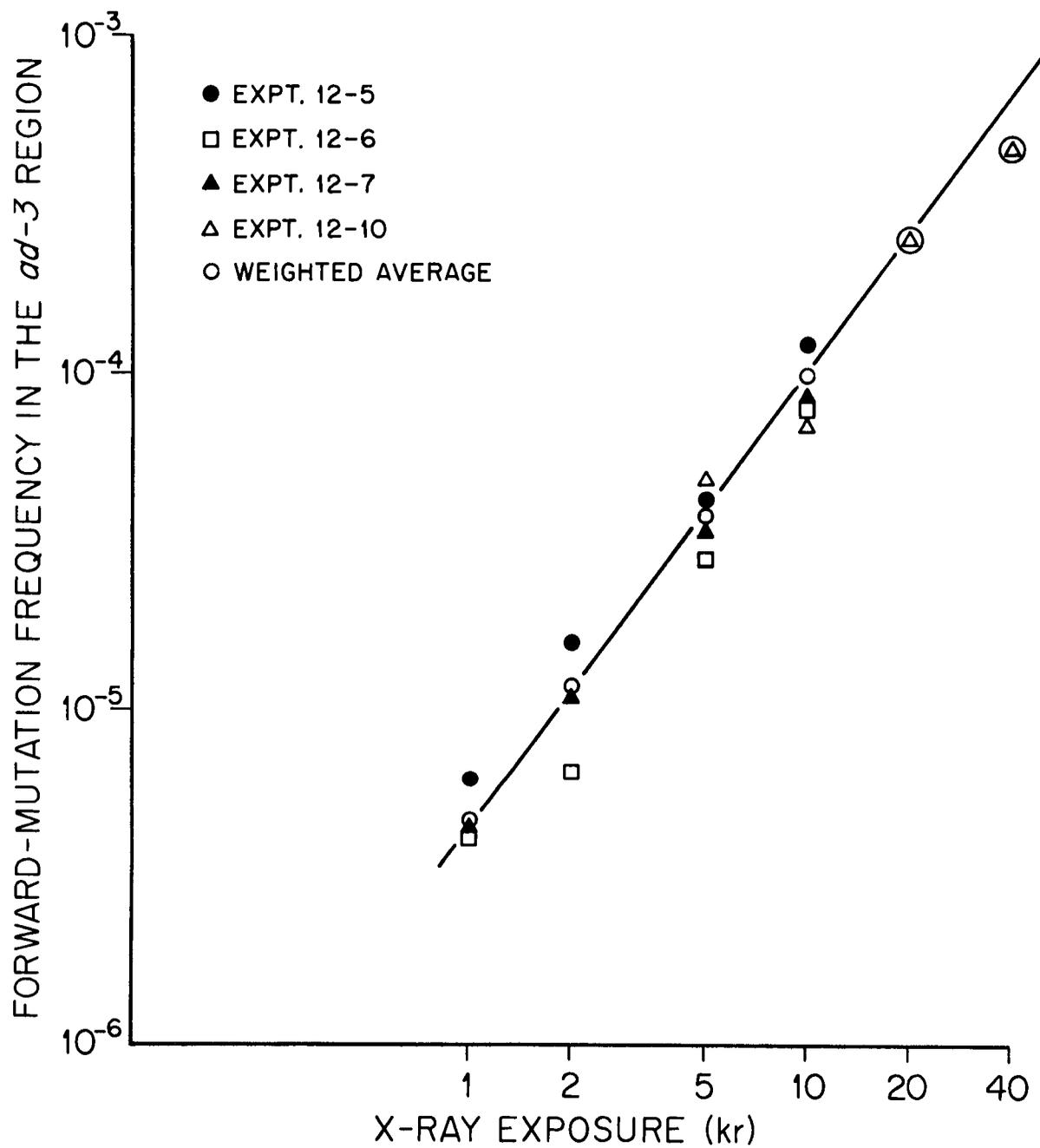


FIG. 2. — Frequency of forward mutations in the ad-3 region after various exposures to 250 kv X rays.

13,731

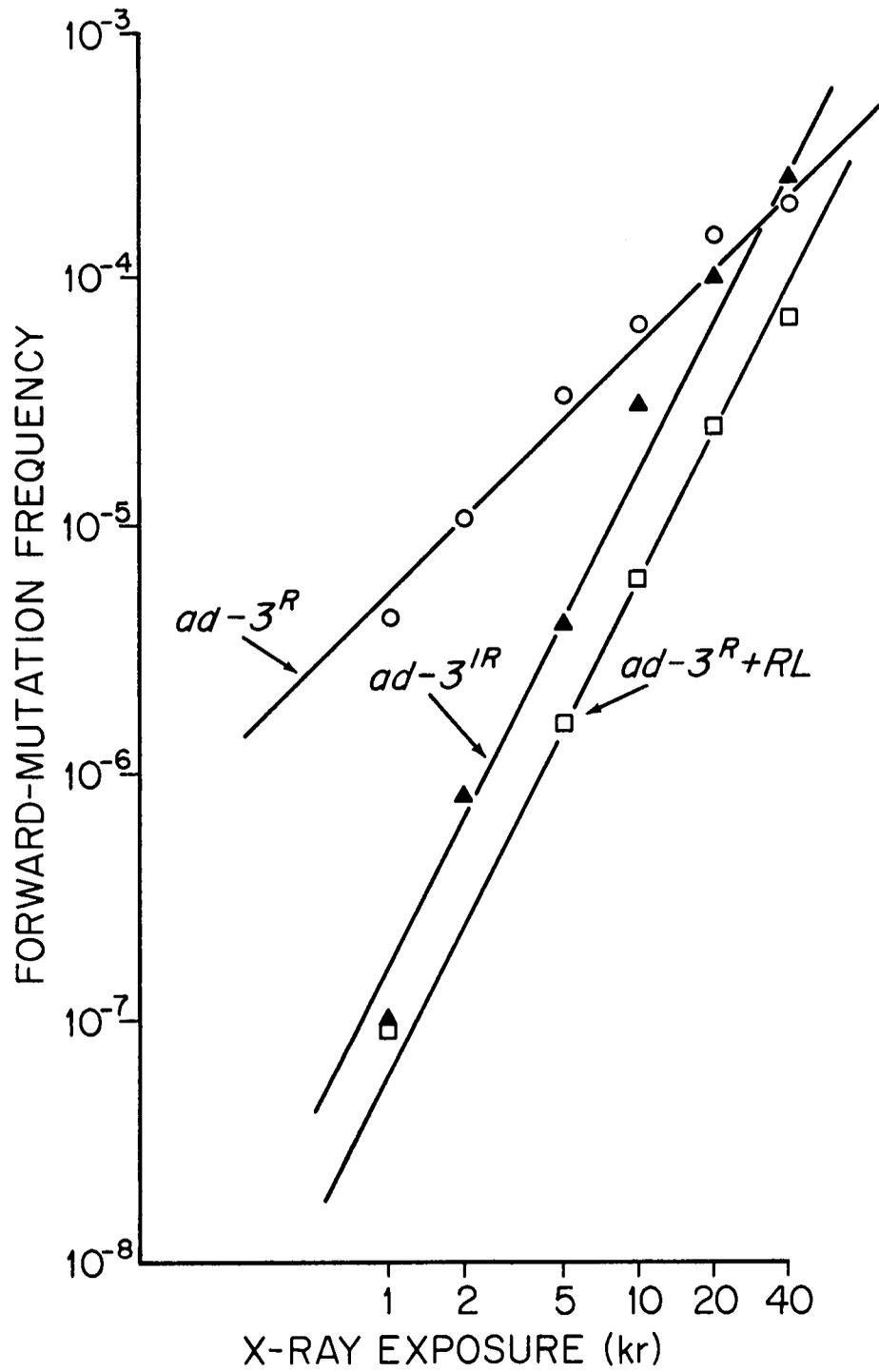


FIG. 3. — Forward-mutation frequencies of various classes of *ad-3* mutations after exposure to 250 kv X rays.

APPENDIX B

Necessary Package Modifications

Problem (1). Screw-on cap placed too close to base plate at bottom of housing units labeled NG-1.

Modification. Increase distance from center of housing opening from 1.00 to 1.50. (Housing on NG-3 packages should be changed in same manner so internal volume of all housings is uniform.) (See sketch No. 1.)

Problem (2). Screw-on caps cannot be screwed on easily.

Modification. Make cap fluted so that it can be gripped more readily. (See sketch No. 1.)

Problem (3). Nylon springs uncoil upon autoclaving.

Modification. Replace with a polycarbonate retaining ring that will hold assembled modules firmly in place inside housing. This retaining ring should be screwed into the mouth of the housing. (See sketch No. 1.)

Problem (4). Polypropylene screen not trimmed around the edges after "welding" to ring.

Modification. Either weld closer to the periphery or trim after welding.

Problem (5). Assembly of polypropylene barrier cumbersome.

Modification. Replace with 1.372 diameter mylar disk 0.005 thick with 3 equally spaced 60° notches to a minor diameter of 1.000". (See sketch No. 2.)

Problem (6). Humidity control inadequate.

Modification. A) Use polypropylene foam 0.25" thick on inside to cover bottom of NG-1 and/or side of NG-3 units. B) Eliminate sponge holder from package.

Problem (7). Endplate material on module.

Modification. A) Make both out of polycarbonate to eliminate distortion. B) Hole description on items P1 and P8 on drawing No. ER470166686 should be interchanged.

*Appendix C taken from "The Neurospora Experiment in the Simulated Space-Flight Tests of April 13 and 19, 1965," F. J. deSerres and B. B. Webber, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Problem (8). External dosimetry inadequate.

Modification. Design new dosimetry pack for glass dosimeters. (See sketch No. 3.)

A) 0.80 diameter polypropylene disk 0.040 with four pairs of radial slots 0.040 diameter 0.240 length equally spaced with outer ends 0.080 from OD of disk.

B) 0.80 diameter mylar disk 0.005 thick with adhesive back with two mylar strips 0.720 x 0.080 centered on disk at right angles to one another.

C) This unit is to be assembled so that the nonadhesive surfaces are directly under the radial slots.

Problem (9). External dosimeter slots not usable.

Modification. The holes should be cleaned out after welding the base plate to the housing.

APPENDIX C



STANDING INSTRUCTIONS - NEUROSPORA FLIGHT PACKAGE

The Neurospora experiment may be divided into two portions (1) preflight operations and (2) postflight operations as described below:

Preflight Operations

- I. Isolation of colonies derived from single heterokaryotic conidia.
- II. Growth and selection of heterokaryotic cultures with 1:1 nuclear ratios.
- III. Cleaning and sterilization of harvesting equipment and of Neurospora flight hardware (package and module components).
- IV. Preparation of jug medium (to be used postflight) and other equipment required for preflight and postflight procedures.
- V. Preparation of conidial suspension.
- VI. Plating of conidial suspension to determine viability and heterokaryotic fraction.
- VII. Preparation of conidial samples on Millipore filters.
- VIII. Insertion of conidial samples into module components.
- IX. Assembly of modules and packages and insertion of dosimeters.
- X. Storage and transport of packages to (simulated) launch site.

(Operations VII, VIII, and IX are performed sequentially so that conidia are exposed to possible contamination for as short a period as possible.)

Postflight Operations

- I. Final sterilization of equipment needed for postflight operations.

- II. Opening of packages and placing of dosimeters into appropriately labeled test tubes.
- III. Removal of selected filters and preparation of conidial suspensions for biological assays.
- IV. Haemocytometer counts of suspensions and estimation of conidial concentrations.
- V. Dosimeter read-outs and estimation of radiation exposures (before or after inoculation of jugs).
- VI. Inoculation of jugs with aliquots of suspensions.
- VII. Incubation of jugs.
- VIII. Cleaning of module components and flight package.
- IX. Harvesting of jugs, isolation of purple colonies, counting of background colonies in aliquots.
- X. Statistical analysis of data to determine survival of heterokaryotic colonies and forward-mutation frequencies.
- XI. Preparation of conidiating cultures from purple colonies.

These operations will now be described. The times indicated will be number of days before departure from ORNL to travel to (simulated) launching site (preflight) or number of days postflight starting with day No. 1 as the first working day on which the flight packages are available at ORNL. It is understood that, depending upon the final plans for turnaround and slippage allowance, the preflight procedures may need to be repeated a number of times with some overlapping in preparations for different flight dates.

All operations involving conidia or unassembled flight package components or any equipment which might come in contact with such components

or conidia require sterile technique and are performed in rooms designed to insure sterile conditions.

Preflight Operations (Details of Individual Steps)

I. Isolation of colonies derived from single heterokaryotic conidia.

12 days preflight. Prepare five 250-ml flasks numbered 1 through 5, each with 100 ml of Fries' minimal medium plus 1.0% sorbose and 0.1% sucrose and 1.5% agar; autoclave 20 min; cool to 45°C in water bath. Take from silica gel stock several crystals with conidia of heterokaryon No. 12, which is composed of 74-OR60-29A (hist-2 ad-3A ad-3B nic-2; ad-2; inos) and 74-OR31-16A (al-2; cot; pan-2). Suspend the conidia in 5-10 ml of water, then add the suspension to flask No. 1, mixing well. Transfer 10 ml from No. 1 to No. 2, mixing well. Pour No. 1 into four or five Petri dishes. Transfer 10 ml from No. 2 to No. 3, mixing well. Pour No. 2 into four or five Petri dishes. Continue through No. 5. Allow plates to gel thoroughly and then incubate plates inverted at 34-35°C for three days.

9 days preflight. Prepare 60-80 flasks (125-ml Erlenmeyer) each with 25 ml of Fries' minimal medium plus 1.5% sucrose and 1.5% agar. Plug with Dispo-plugs and autoclave 20 minutes with slow exhaust and allow to gel at room temperature (23-25°C). Select from those plates which are not too crowded single colonies which have a large, spreading, and well-formed morphology but not those with the morphology of the colonial-temperature-sensitive mutant. Place single colony isolates in the center of the agar in the flasks (one isolate per flask). Incubate the flasks for one day at 34-35°C.

II. Growth and selection of suitable Neurospora cultures and production of conidia.

8 days preflight. Examine flasks; mark those in which spreading growth occurred (with mycelium covering thoroughly most of the surface of the agar). Incubate colonies for seven days more at 23-25°C in laboratory light.

1 day preflight. Select flasks with bright orange conidia and preferably those which showed spreading growth at 8 days preflight. In the past, eight flasks have provided more than 5×10^9 conidia after harvesting, cottonfiltering, etc.

III. Cleaning and sterilization of Neurospora packages and module components

1-3 days preflight. Disassemble Neurospora packages and modules. Remove glass rod dosimeters from all dosimeter compartments. Wash all components in detergent solution. Rinse thoroughly with distilled water. Shake off excess water and blot dry with paper towels. Prepare module and package components for sterilization by autoclaving as follows:

In paper bags - one package assembly, one cover (P1), and one gasket (P9) per bag; fold top down twice and staple twice.

Petri dishes (pyrex, 2 cm x 14 cm) - ten polypropylene rings (P6) each with retainer rings (P2) inserted, one end plate (P8), one sponge (P10), and three screws (P5) per Petri dish. Place objects into Petri dish separated by layers of filter paper.

Fasten top of Petri dish to bottom in two places with temperature indicator tape.

Petri dishes (pyrex, 2 cm x 14 cm) - Place ten polypropylene barriers per dish in layers separated by filter paper. Tape as above.

Petri dishes (pyrex, 2 cm x 14 cm) - twenty to thirty Millipore

filters (type RAWP 02500) per Petri dish in layers separated by filter paper. Be sure that filters are not in contact with one another. Tape as above.

1 day preflight. Autoclave above items at 250°C for 30 minutes with fast exhaust; allow to cool and dry at room temperature.

IV. Preparation of jug medium (to be used postflight) and other equipment required for preflight and postflight operations.

1-3 days preflight. Prepare for autoclaving:

Platinum filters (one per culture) in paper bags (one per bag).

Test tubes of glass beads (3 mm diameter; about 300-450 per tube); with cap-type closures; one tube per culture.

Two 250-ml Erlenmeyer flasks with cap-type closure.

Two 1000-ml flat-bottomed Florence flasks with magnetic stirring bars and cap-type closures.

Millipore harvesting apparatus (using large vacuum flasks with side-arm).

Two Buchner funnels with thick pads of cotton in filter portion and cotton inserted into side-arm; cover filter portion and side-arm with aluminum foil.

Graduated cylinders - two 100 ml, two 250 ml, and two 1000 ml; cover open ends with aluminum foil.

Pipettes (0.2 ml, 1.0 ml, and 10.0 ml) with different sizes in different marked cans or pyrex cylinders.

1-2 days preflight. Autoclave above items (250°C) for 30 minutes plus fast exhaust; cool to 4-6°C. Autoclave one test tube of water for each culture to be harvested (10-12 ml water per tube) and four 1000-ml flasks

each with 500-700 ml water. Use 25 minutes plus slow exhaust. Cool this to 4-6°C.

Place lithium fluoride-teflon disk dosimeters into a Petri dish between layers of filter paper. Sterilize in dry oven at 150°C for two hours. Cool to room temperature.

1-3 days preflight. Prepare flasks of plating medium as described in preflight section VI. Autoclave at 1 day preflight per section VI.

1-7 days preflight. Prepare jug medium, dispense it into jugs and sterilize by autoclaving 60 minutes with slow exhaust cycle per laboratory instructions. Jugs must be cooled at 25°C for two days and then brought to 30°C (which requires at least 24 hours at 30°) before inoculation. Flasks of sorbose solution (150 g. sorbose dissolved in 500 ml sterile water in 1000-ml flasks) which are each added to a jug at the time of inoculation must also be prepared and autoclaved (10 minutes plus slow exhaust) at some convenient time before inoculation.

V. Preparation of conidial suspension.

Select cultures on the basis of (1) spreading (noncolonial) growth at 35°C after one day incubation (at 8 days preflight) and (2) deep orange conidial color at harvesting time (1 day preflight). Eight cultures should provide more than 5×10^9 conidia. Add glass beads to a culture flask; shake flask vigorously so that glass beads reduce the strands of conidia to individual conidia. Add 10-12 ml ice-cold water; shake with a circular horizontal motion to suspend conidia and further break strands. Pour suspension through a platinum filter into a 250-ml flask. Repeat with next culture, pouring through a separate platinum filter into the same flask.

Strain the suspension derived in this way from all cultures used through a premoistened thick cotton pad in a Buchner funnel. Strain the resulting suspension through a second premoistened cotton pad. This procedure should give a conidial suspension of cells of approximately the same size. Make a 1/100 dilution tube (0.1 ml suspension plus 9.9 ml water) and perform haemocytometer counts of about 400 conidia in each of two separately pipetted fields. Estimate conidial concentration. Make up from this suspension a flask containing 5×10^9 conidia in 100 ml of suspension (5×10^6 conidia per ml) and a magnetic stirring bar. (The total volume required may actually be greater or smaller depending upon the number of packages needed.) Check the final conidial concentration by additional haemocytometer counts, adjusting as necessary. (Note: From the time the cold water is first added to the culture flasks until the packages are released to G. E. personnel for attachment to shield and vehicle, the conidia are kept as close to ice-water temperature as is consistent with the required operations.)

VI. Plating of conidial suspension to determine survival and heterokaryotic fraction.

Prepare (before day No. 1 preflight) four 250-ml flasks each containing 100 ml of Westergaard's minimal medium plus 1.0% sorbose and 0.05% fructose and 0.05% glucose and 1.5% agar. Prepare four 250-ml flasks each containing 100 ml of the above basic medium supplemented with DL-histidine-HCl·H₂O (100 γ /ml), adenine sulfate (100 γ /ml), nicotinamide (10 γ /ml), inositol (8 γ /ml) and calcium pantothenate (γ γ /ml). Autoclave at day No. 1 preflight for 20 minutes plus slow exhaust and cool at 45°C in water bath.

Add 0.1 ml of suspension to 9.9 ml of water; stir well (with Vortex mixer); transfer 0.1 ml from first tube to a second tube of 9.9 ml water; stir well. Add 2 ml from the second tube to each of the flasks of minimal medium and add 1 ml from the second tube to each of two flasks of supplemented medium. Stir well. Pour each flask into five Petri dishes. Allow medium to gel; incubate at 30°C inverted. Repeat this procedure so that a total of four flasks of minimal medium and four flasks of supplemented medium are derived from two separate dilution series and plated.

Count minimal plates after three days and supplemented plates after four days. Compute viability on the basis of 500 haemocytometer-countable conidia per series of supplemented plates. Estimate viable heterokaryotic conidia on the basis of 1000 conidia per series of five minimal plates.

VII. Preparation of conidial samples on Millipore filters (P3).

Standard Millipore equipment for 25-mm diameter filters is used for deposition except that the fritted glass surface which receives the filter is fitted onto a larger vacuum flask with side-arm. Place the flask with the adjusted conidial suspension into an ice bath in a plastic pan and set pan and flask over a magnetic stirrer. Permit magnetic stirrer to resuspend the conidia and make a uniform suspension. Using blunt forceps provided by Millipore, place one filter (type RAWP 02500 = G.E.'s P3) onto the fritted glass surface; place the glass cylinder on top and clamp it there. Pipette ten ml of suspension into the glass cylinder and remove the water by vacuum through side-arm. Break the vacuum at the end of the vacuum tubing farthest from the flask. Remove clamp and cylinder so the filter with conidia may be removed easily. Continue with operation VIII.

VIII. Insertion of conidial samples on Millipore filters (P3) onto screens in module rings (P6).

When operation VII has been completed, remove a retaining ring (P2) from a module ring (P6). Remove moist filter with conidia (P3) from fritted glass surface of harvesting equipment. Place filter with conidia up onto the more indented surface of the screen, replace the retainer ring (P2) which holds the filter in place.

Before the first ring in a stack of ten only, insert three screws (P5) into holes in end plate (P8) so that the heads are countersunk and place the end plate onto a sterile surface. Place the module ring (P6) with filter (P3) conidia side up and retaining ring (P2) over the screws and into place on the inner surface of the end plate (P8). Cover with a polypropylene barrier (P7) and snap the ring (P6) into place.

Repeat operations VII and VIII alternately until the stack of ten rings is complete.

IX. Assembly of modules and packages and insertion of dosimeters.

Insert lithium fluoride-teflon disk dosimeters into the stack as required.

Place package cover (P1) with outside surface down upon a sterile surface. Put gasket (P9) into place on cover. Place lithium fluoride disk dosimeters (three) on cover (P1) and invert stack of ten rings onto the cover so screws and threads line up. Tighten screws with torque-limiting screw driver.

Moisten sponge (P10) and place this encircling the module stack at approximately the center (ring No. 5-6) of the stack.

Place lithium fluoride-teflon disk dosimeters (three) on the end plate (P8) and place the flight package over the module. Screw the cover with attached module into the package to make a tight seal. Store the assembled package at 2-6°C until time to fasten it to the vehicle. Expose conidia to possible contamination for as short a time as possible. Repeat VII and VIII alternately and IX after ten rings are prepared.

X. Storage and transport of packages to (simulated) launch site.

Transportation equipment consists of one altered cosmetics case ("case") fitted with a tap at the bottom and one copper box and lid ("box") fitted with gaskets and locks to permit a water-tight seal.

Precool box and lid to 2-6°C. Place flight packages into box on a cushion of paper towels. Cover and seal with lid. Place box into case and fill space between inside of case and outside of box with crushed ice. During transport replenish ice supply as needed, draining excess water from case through tap at bottom of case. Upon arrival at launching site, place the case with box into 2-6°C facility. Remove flight packages from box at latest time possible before fastening package to vehicle.

Standing Instructions for attachment of packages to bracket and plate, etc., are being prepared at Ames.

Postflight Operations

Flight packages are removed from vehicle per Standing Instructions being prepared at Ames and kept at ice-water temperatures until they are

opened and the filters removed. Day No. 1 is the first working day on which the packages are available at ORNL.

I. Final check and sterilization of equipment needed for postflight operations.

Preparation of most of the equipment for postflight operations is described in preflight section IV. Materials required immediately include the following:

Pipettes - 0.2, 1.0, 2.0, and 10.0 ml with different sizes in separately marked or transparent containers.

Forceps (blunt, Millipore)

Alcohol (95% and 70%) and flame for sterilizing forceps.

Test tubes (20 x 150 mm) - one for each filter of conidia to be used and one for each group of dosimeters. Use aluminum cap type closure.

Water - 500 ml in 1000-ml flask.

Jugs with medium - 12-liter capacity with about 10 liters medium; fitted with cotton plug, glass bubbling tube, and glass wool filter. (Number of jugs varies with experiment; as many as 100 may be required.)

Sorbose solution in 1000-ml flasks (150 g dissolved in 500 ml sterile water and autoclaved 10 minutes plus slow exhaust); one flask required for each jug.

Compound microscope, haemocytometer slide, hand tally.

Jugs will have been autoclaved on the day they were prepared. Preparing twenty jugs constitutes a day's work. Sorbose flasks should have been prepared and autoclaved on day No. 1 or the preceding day. Autoclave

test tubes and water and add 10 ml water to each of the tubes for conidia on day No. 1 or preceding day. Keep these at 2-6°C. Tubes for dosimeters should be dry.

II. Opening of packages and placing of dosimeters into appropriately labeled test tubes.

Sterilize the outside surface of the flight package, especially the package cover (P1) and the area near the gasket (P9), with 70% alcohol, allowing excess to evaporate to dryness. Unscrew package cover (P1) with attached module and place this on a sterile surface with the outside surface of the package cover down. Transfer the (three) lithium fluoride-teflon disk dosimeters from the end plate (P8) to a test tube marked with "r" (for "rear") plus an arbitrary numerical designation for the package. Unscrew the screws (P5) and remove module from cover (P1). Transfer dosimeters from inside of cover to tube marked "f" (for "front") and arbitrary package designation. Break the stack of rings (P6) so as to remove other dosimeters in the stack and transfer these to tubes marked with the filter numbers (e.g., "3-4" for dosimeters between the third and fourth filter from the front) and arbitrary package designation.

III. Removal of selected filters and preparation of suspensions of conidia from the filters.

Break stack of rings in a position appropriate for removing one of the filters. Remove polypropylene barrier (P7) and retainer ring (P2) from one ring (P6) with sterile forceps; lift filter (P3) with conidia from ring and insert it into a test tube containing 10 ml of sterile ice-cold water. Shake with Vortex mixer to remove conidia from the filter. Some time may be required to soak the conidia loose. When

conidia have been removed from the filter (i.e., when filter appears white), remove and discard the filter. Keep tubes at ice-water temperature with ice bath before and after adding the conidia. Finish harvesting conidia from remaining filters in open package quickly so as to maintain moistness and low temperature of conidia.

IV. Haemocytometer counts of suspensions and estimation of conidial concentrations.

After completing each package or after repeating this procedure with other packages until conidia from the whole series of packages are suspended, perform haemocytometer counts on two 10^{-4} ml aliquots from each suspension. Estimate conidial concentration for each suspension.

V. Dosimeter read-outs and estimation of radiation exposures (before or after inoculation of jugs).

If arrangements are made to estimate radiations exposures before inoculating jugs, then thermoluminescence readings must be obtained from the lithium fluoride-teflon disk dosimeters on postflight day No. 1 after conidia are harvested. We may decide to base inoculation volumes on assumed dosage, in which case the thermoluminescence readings could be deferred until work load is lighter.

VI. Inoculation of jugs with aliquots of suspensions and addition of sorbose solution.

On the basis of haemocytometer counts, estimates of viable heterokaryotic fraction of conidia (based on preflight procedure VI), and expected percentage survival based on assumed or measured radiation exposure, estimate volume of each suspension required to contain 8×10^5 heterokaryotic viable conidia. Inoculate each jug as described

subsequently. Remove paper bag covering plug and lip; lift plug from lip of jug and add sorbose solution from one of the 1000-ml flasks; replace plug into neck of jug; mix sorbose and jug contents by moving jug with circular horizontal motion; stir suspension with Vortex mixer and take into a pipette the appropriate amount of that suspension; while the medium is still circulating, hold the plug several inches above the lip of the jug and add contents of the pipette to the jug contents; replace the plug; replace the paper bag; record on paper the jug number, the suspension designation, and the volume of suspension added.

VII. Incubation of jugs.

Place jugs in dark or red light at 30°C and attach rubber tubing from air source to glass wool filter which is attached to the bubbling tube. Adjust the air flow to 0.04 (arbitrary units on standard flow-meter). Observe periodically to see that bubbling occurs in all flasks and after three or four days to see whether colony morphology and concentration appear normal. Jugs must incubate in the dark or red light at 30°C during the full incubation period of six days.

VIII. Cleaning of module components and flight packages.

Finish disassembly of packages and modules, discarding any filters not required. Remove and store in paper envelopes glass dosimeters (if used). Wash all components in water and laboratory detergent. Rinse thoroughly with distilled water. Shake off excess water and blot dry with paper towels; finish drying in 60°C oven. Assemble packages and modules for storage.

IX. Harvesting of jugs, isolation of purple mutants, counting of background colonies.

After six days of incubation disconnect air line, remove cotton plug and bubbling tube from a jug, mix jug contents by moving jug with horizontal circular motion, then pour 1500 ml into a sterile graduated cylinder. Place a sterile 10-ml cup (e.g., test tube cap) into a white sterile photographic developing pan (55 × 45 cm) and, before the colonies settle in the cylinder, pour contents into the pan and a representative 10-ml aliquot into the 10-ml cup. Pour contents of cup into a 20 × 150 mm test tube, rinsing residual colonies from cup with 95% alcohol. Add about 10-15 ml to the colonies to preserve them for subsequent background colony counts.

With sterile forceps, pick all purple colonies from the pan, counting them, and place them into a Petri dish of sterile water. Discard white colonies and medium and pour second 1500-ml aliquot. (Note that the sixth aliquot will normally contain more than 1500 ml.) Background colonies in the 10-ml samples are counted by machine. For each sample of 1500 ml (or more) record the necessary information (sample volumes, number of purple colonies, number of background colonies in 10 ml sample, etc.) on standard data sheets.

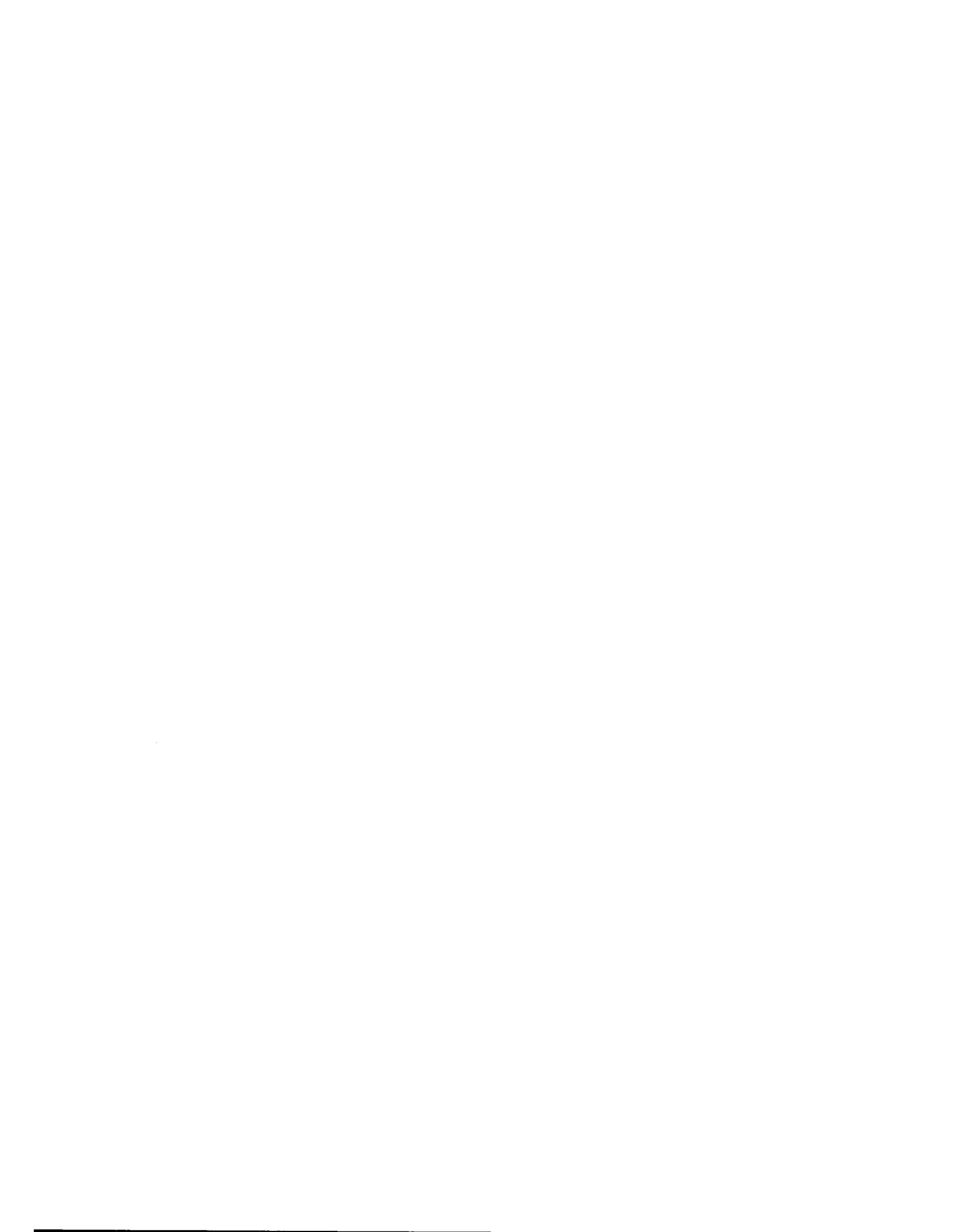
X. Estimation of survival and forward-mutation frequencies.

Survival is determined by a comparison of the estimates of background colony counts from jugs containing treated conidia with those counts from jugs containing untreated conidia. Forward-mutation frequencies are determined from the total number of purple colonies/total number of background colonies for each treatment series.

XI. Preparation of conidiating cultures from the purple colonies.

Remove white sectors from each purple colony and rinse in distilled water. Insert each colony into a 10 × 75 mm test tube containing a 1-ml agar slant of Fries' medium plus 1.5% sucrose plus 100 γ /ml adenine sulfate plus 10 γ /ml nicotinamide plus 250 γ /ml L-arginine HCl. Allow tubes to incubate about seven days at 25°C so that colonies each grow to form conidiating cultures. Analytical tests designed to characterize each mutational event are carried out subsequently, but will not be described here.

APPENDIX D



FTI 239952 C

C REVISION
22 Nov 1966

Rev approved by _____

TASK A - EXPERIMENTER FTI NEUROSPORA

TimeVerified1.0 Flight Test Instructions for Loading and
Assembling Neurospora Modules at ORNL

T-104 Hrs.

- 1.1 Obtain flask containing a magnetic stirring bar and a suspension of five million conidia per ml. In the sterile room, place this flask into an ice bath in a plastic pan over a magnetic stirrer. Permit magnetic stirrer and bar to resuspend conidia and make a uniform suspension. _____
- 1.2 Use standard Millipore equipment for 25-mm diameter filters with a 500 ml. vacuum flask; employ sterile technique. Use blunt Millipore forceps to place a filter (G.E.'s P3; Millipore's RAWP 02500) onto the fritted glass surface. Place glass cylinder on top and clamp it there. Pipette ten ml. of suspension into the glass cylinder. Apply just enough vacuum to the flask to remove the water from the cylinder without drying the filter excessively. _____
- 1.3 Insert three screws (P5) into holes in end plate (P8) so that the heads are counter-sunk and put the end plate onto a sterile surface with screws pointing up. _____
- 1.4 Remove a retainer ring (P2) from a module ring (P6). Place three sterile 5-mil lithium fluoride teflon disk dosimeters onto the more indented surface of the module ring screen. Place the moist filter (from procedure 1.2) with conidia up, onto the dosimeters. Moisten retainer ring by dipping it into sterile water and blotting on sterile filter paper. Put retainer ring into position on module ring. _____
- 1.5 Place the module ring with filter side up over the screws and into place on the stack. Cover the module ring with a polypropylene barrier (P7) and snap the module ring into place. _____
- 1.6 Repeat operations 1.2, 1.4, and 1.5 consecutively but with dosimeters in selected module rings only. (Usually numbers 1, 5 and 10 or numbers 1, 3, 5, 7 and 10 numbering from the cap) until the stack of ten disks is completed. _____

FTI 239952 C

C REVISION

22 Nov. 1966

Rev, approved by _____

TASK A (continued)

TimeVerified

- 1.7 Place plastic plug (obtained by cutting an identi-plug, size B, or equivalent in half perpendicular to the long axis) into cavity in assembly cover (P1). Be sure that gasket is in place surrounding the cover. Add 0.5 ml. of sterile water to plug and allow this to soak in. _____

Invert stack of disks (from paragraph 1.6) over cap so that screws enter the threaded holes in the cap. Torque screws to 3 ± 1 in oz. _____

- 1.8 Repeat procedures 1.2 through 1.6 for all modules required for the test or flight. _____

- 1.9 Transportation equipment consists of altered cosmetics cases ("cases") which accommodate stainless steel boxes ("boxes"). Pre-cool sterile boxes to 2-6°C. Screw modules as they are assembled into holes in boxes. As each is filled with five modules, tighten screw latches to seal box and place it into a case. Fill the space around the box with crushed ice. During transport, replenish ice supply as needed. At launch or test site place cases containing boxes into 2-6°C facility until time to couple housings into modules. _____

2.0 Washing and Sterilizing Neurospora Housings

T-25 Hrs.

- 2.1 Obtain six housings from bonded storage. _____

- 2.2 Prepare a dilute solution of Haemosol or 7X detergent. _____

- 2.3 (Thermistors are somewhat fragile; do not touch them with brush during washing). Rinse housings inside and outside with detergent solution, brushing very lightly with a soft bristled brush. Allow detergent solution to drain out. _____

- 2.4 Rinse each housing, inside and outside, six times with clean distilled water. To rinse the inside, fill with distilled water, fresh from the source, discarding the water after each such rinse. Allow the housings to drain well between rinses. _____

FTI 239931 B

2.4 (Continued)

Six housings rinsed first time. _____

Six housings rinsed third time. _____

Six housings rinsed fourth time. _____

Six housings rinsed fifth time. _____

Six housings rinsed sixth time. _____

2.5 Prepare each housing for autoclaving by placing it into a paper bag, folding the top down twice then stapling the fold, and by placing this bag into a second bag, folding the top down twice then stapling the fold. (Each housing should be double-bagged separately.) _____

2.6 Autoclave the double-bagged housings at 250° F. twenty minutes with fast exhaust and 10-15 minutes of drying. _____

2.7 Deliver these clean, sterile, double-bagged housings to bonded storage or commence coupling procedure. _____

3.0 Flight Test Instructions for Coupling Modules with Housings

3.1 Turn on sterile air supply for clean room about 30 minutes before module and housings are to be coupled. _____

3.2 Clean and sterilize table in clean room and adjacent wall area with cloth and 70% ethanol. _____

3.3 Remove aluminum box containing modules from carrying case (i.e., modified cosmetics case) and dry the aluminum box well on all sides with cloth or paper toweling. (Do this outside of the clean room). _____

3.4 Inside the clean room, sterilize the outer surface of the (aluminum) module box with 70% ethanol and cloth and place the module box on the table. _____

FTI 239952 B

TASK A (continued)

<u>Time</u>		<u>Verified</u>																												
T-775	3.5 Obtain the double-bagged housings from bonded storage. Remove the outer bags at the entrance to the transfer room and place each inner bag, containing a sterile housing, onto the table.	_____																												
T-770	3.6 During the coupling, keep the module box lid closed as much as possible to preserve sterility. Remove a housing from a paper bag and insert a module. Torque the module into the housing until the rubber gasket meets the housing without gaps. Do not over torque. Record the number of other designation for the module and its housing. Repeat for each housing module pair.	_____																												
	<table border="0" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th></th> <th style="text-align: center;"><u>Housing Designation</u></th> <th style="text-align: center;"><u>Module Designation</u></th> <th></th> </tr> </thead> <tbody> <tr> <td>First coupling</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> </tr> <tr> <td>Second coupling</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> </tr> <tr> <td>Third coupling</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> </tr> <tr> <td>Fourth coupling</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> </tr> <tr> <td>Fifth coupling</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> </tr> <tr> <td>Back-up Pkg.</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> <td style="text-align: center;">_____</td> </tr> </tbody> </table>		<u>Housing Designation</u>	<u>Module Designation</u>		First coupling	_____	_____	_____	Second coupling	_____	_____	_____	Third coupling	_____	_____	_____	Fourth coupling	_____	_____	_____	Fifth coupling	_____	_____	_____	Back-up Pkg.	_____	_____	_____	
	<u>Housing Designation</u>	<u>Module Designation</u>																												
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Third coupling	_____	_____	_____																											
Fourth coupling	_____	_____	_____																											
Fifth coupling	_____	_____	_____																											
Back-up Pkg.	_____	_____	_____																											
T-760	3.7 After the last module has been removed from the aluminum module box, close the module box tightly so that it can be kept sterile for use after the test or flight without further sterilization.	_____																												
	3.8 Turn off the supply of sterile air.	_____																												
T-660	3.9 Deliver assemblies to the G.E. Assemblers at appropriate time. (Five (5) Flight and One (1) back-up package)	_____																												

APPENDIX E



VIBRATION QUALIFICATION TEST
#201 Hardware

May 26 - June 10, 1966

Heterokaryotic survivals from plating data

Test Position	Conidia exposed to 5000 R before test		Unirradiated conidia		Conidia exposed to 5000 R after test	
	Proportion of all conidia	Percent of original heterokaryotic fraction (0.2217)	Proportion of all conidia	Percent of original heterokaryotic fraction (0.2217)	Proportion of all conidia	Percent of original heterokaryotic fraction (0.2217)
A 809	.0679	30.63	.1720	77.58	.0754	34.01
A 816	.0828	37.35	.1773	79.97	.0860	38.79
A 817	.0998	45.02	.1921	86.65	.1146	51.69
A 818	.0796	35.90	.1847	83.31	.1115	50.29
A 819	.0924	41.68	.1932	87.14	.1072	48.35
Average	.0845	38.11	.1839	82.95	.0989	44.61

VIBRATION QUALIFICATION TEST
#201 Hardware

May 26 - June 10, 1966

Heterokaryotic survival and mutation data for individual jugs

Test Position	Treatment	Mutation frequency X 10 ⁶		Heterokaryotic survival		
		Individual jugs	Mean	Individual jugs	Mean	Percent of original Heterokaryotic fraction (0.2217)
A 809	5000 R before test	164.3	162.5	.0964	.0985	44.43
		171.0		.0918		
		167.6		.0896		
		147.0		.1161		
	Unirradiated	2.0	0.7	.2011	.1916	86.42
		0		.1718		
		0		.2020		
	5000 R after test	153.5	155.1	.0831	.0952	42.94
		154.3		.1183		
157.5		.0843				
A 816	5000 R before test	144.2	188.2	.0464	.0618	27.88
		186.0		.0620		
		200.7		.0802		
		221.8		.0584		
	Unirradiated	0	0	.1207	.1577	71.13
		-		.1946		
		0		.1575		
	5000 R after test	184.7	154.8	.0770	.0826	37.26
		142.3		.0910		
137.4		.0797				
A 817	5000 R before test	225.0	189.3	.0822	.0849	38.29
		187.1		.0897		
		155.9		.0828		
		0		0		
	0	.1333				
	0	.1422				
	5000 R after test	139.4	134.6	.0938	.0877	39.56
		123.2		.0857		
		141.3		.0837		
A 818	5000 R before test	182.7	182.2	.0876	.0818	36.90
		182.9		.0860		
		158.7		.0869		
		204.6		.0667		
	Unirradiated	0	0.4	.1522	.1673	75.46
		0		.1755		
		1.1		.1743		
	5000 R after test	140.4	141.1	.1036	.0876	44.02
		146.0		.0920		
137.0		.0971				
A 819	5000 R before test	154.4	160.6	.0776	.0783	35.32
		-		.0828		
		131.5		.0793		
		196.0		.0734		
	Unirradiated	0	0	.1706	.1507	67.97
		0		.1289		
		0		.1525		
	5000 R after test	149.2	143.5	.0921	.0901	40.64
		135.3		.0869		
146.0		.0912				
Mean mutation frequency X 10 ⁶ for pre-irradiated conidia		176.7				
Mean mutation frequency X 10 ⁶ for unirradiated conidia		0.2				
Mean mutation frequency X 10 ⁶ for post-irradiated conidia		145.8				
Mean Heterokaryotic survival for pre-irradiated conidia				.0811		36.58
Mean Heterokaryotic survival for unirradiated conidia				.1617		72.94
Mean Heterokaryotic survival for post-irradiated conidia				.0906		40.87

THERMAL VACUUM QUALIFICATION TEST #I
#201 Hardware

June 8 - 17, 1966

Heterokaryotic survivals from plating data (no jugs were inoculated)

Test Position	Conidia exposed to 5000 R before test		Unirradiated conidia	
	Proportion of all conidia	% of original Heterokaryotic fraction (0.2272)	Proportion of all conidia	% of original Heterokaryotic fraction (0.2272)
A 809	.1070	47.10	.2008	88.38
A 816	.1223	53.83	.1674	73.68
A 817	.1274	56.07	.1894	83.36
A 818	.1154	50.79	.1959	86.22
A 819	.1341	59.02	.2336	102.82
Average	.1212	53.35	.1974	86.88

NOTE: This was the experiment which went for the full three days, but in which environmental controls were inadequate.

COMMENTS:

Interval from module preparation to inoculation - 8 days.

Survival is slightly higher than estimated for 201 vibration tests in spite of longer incubation time for thermal/vacuum test.

Controls outside of the test area should be assayed.

THERMAL-VACUUM QUALIFICATION TEST #2
#201 Hardware

July 14 - 22, 1966

Heterokaryotic survival from plating data (no jugs used)

Test Position	Conidia exposed to 5000 R before test		Unirradiated conidia	
	Proportion of all conidia	% of original Heterokaryotic fraction (0.2430)	Proportion of all conidia	% of original Heterokaryotic fraction (0.2430)
A 809	.1539	63.33	.2197	90.41
A 816	.1180	48.56	.1796	73.91
A 817	.1280	52.67	.2673	110.00
A 818	.1498	61.65	.1569	64.57
A 819	.1945	80.04	.2195	90.33
Average of experimental conidia	<u>.1488</u>	<u>61.23</u>	<u>.2086</u>	<u>85.84</u>
Control #1 (room temp.)	.1625	66.87	.2279	93.79
Control #2 (refrig.)	.1173	48.27	.2432	100.08
Average of controls	<u>.1399</u>	<u>57.57</u>	<u>.2356</u>	<u>96.95</u>

NOTE: This was the thermal vacuum sequence which was abated often one day.

COMMENTS:

Interval from module preparation to inoculation - 8 days.

Survival about the same as previous thermal vacuum run; lower than

controls enclosed in housings from Ames Biosatellite Test.

THERMAL VACUUM QUALIFICATION TEST #3
#201 Hardware

July 25 - August 1, 1966

Heterokaryotic survival from plating data

Test Position	Conidia exposed to x-rays* before test		Unirradiated conidia	
	Proportion of all conidia	% of original Heterokaryotic fraction (0.1768)	Proportion of all conidia	% of original Heterokaryotic fraction (0.1768)
A 809	.1262	71.38	.1808	102.26
A 816	.1029	58.20	.1695	95.87
A 817	.1089	61.60	.1842	104.19
A 818	.1153	65.21	.1509	85.35
A 819	.1096	61.99	.1585	89.65
Average of experimental conidia	<u>.1126</u>	<u>63.69</u>	<u>.1688</u>	<u>95.48</u>
Control (room temp.)	.1276	72.17	.1301	73.59
Control (refrig.)	.1467	82.98	.1591	89.99
Average control	<u>.1372</u>	<u>77.60</u>	<u>.1446</u>	<u>81.79</u>

* Because of a technical error, the x-ray exposure in this experiment is not known but is thought to be about 3250 R. Accordingly, estimates of mutant variation from sample to sample could be obtained, but not quantitative dose-exposure relationships.

THERMAL VACUUM QUALIFICATION TEST #3
#201 Hardware

July 25 - August 1, 1966

Heterokaryotic survival and mutation data for individual jugs

Test Position	Treatment	Mutation frequency X 10 ⁶		Heterokaryotic survival		
		Individual jugs	Mean	Individual jugs	Mean	Percent of original Heterokaryotic fraction (0.1768)
A 809	x-rays* before test	17.6 27.7	22.7	.1076 .0986	.1031	58.31
	Unirradiated	0.9 0 1.0	0.6	.1643 .1607 .1522	.1591	89.99
A 816	x-rays before test	15.8 24.5 33.6	24.6	.1102 .1109 .1102	.1104	62.44
	Unirradiated	0.8 0 0	0.3	.1508 .1608 .1509	.1542	87.22
A 817	x-rays before test	24.3 17.5 18.3	20.0	.1232 .1121 .1134	.1162	65.72
	Unirradiated	0 0.9 3.3	1.4	.1484 .1581 .1312	.1459	82.52
A 818	x-rays before test	18.4 26.5	22.5	.1477 .1283	.1380	78.05
	Unirradiated	0 0 1.0	0.3	.1436 .1533 .1264	.1411	79.81
A 819	x-rays before test	29.5 22.4 29.1	27.0	.1005 .1190 .1056	.1082	61.20
	Unirradiated	1.0 1.2 0	0.7	.1428 .1227 .1461	.1372	77.60
72° F c.t.	x-rays before test	24.0 26.8 15.3	22.0	.1310 .1315 .1232	.1286	72.74
	Unirradiated	0 0.8 1.1	0.4	.1518 .1899 .1521	.1646	93.10
28° F	Unirradiated	1.8 2.3 0.7	1.6	.1303 .1524 .1656	.1494	84.50
	Unirradiated	0 0.8 0	0.3	.1658 .1785 .1955	.1799	1.8
	Means for jugs with irradiated conidia		23.8		.1179	66.69
	Means for jugs with unirradiated conidia		0.7		.1546	87.44

* X-ray exposure ill-defined because of technical error.

SUMMARY AND COMMENTS ON THERMAL VACUUM QUALIFICATION (201)
Test #3

Interval from module preparation to inoculation 7 days

Estimated average survival of unirradiated in test - jugs . . . 87.44
plates . . 95.48

Uncertainty about x-ray exposure makes mutation frequencies irrelevant.

Jug to jug variation seems low.

VIBRATION ACCEPTANCE TEST

#301 Hardware

June 30 - July 5, 1966

Heterokaryotic survival from platings (no jugs inoculated)

Test Position	Conidia exposed to 5000 R before test		Unirradiated conidia		Conidia exposed to 5000 R after test	
	Proportion of all conidia	Percent of original heterokaryotic fraction (0.2331)	Proportion of all conidia	Percent of original heterokaryotic fraction (0.2331)	Proportion of all conidia	Percent of original heterokaryotic fraction (0.2331)
A 809	.1097	49.17	.1670	74.85	.1198	53.70
A 816	.0918	41.19	.1528	68.49	.0812	36.40
A 817	.0790	35.41	.2083	93.37	.1229	55.09
A 818	.0944	42.31	.1810	81.13	.0920	41.24
A 819	.1127	50.52	.1819	81.53	.1016	45.54
Average	<u>.0975</u>	<u>43.70</u>	<u>.1782</u>	<u>79.87</u>	<u>.1035</u>	<u>46.39</u>

COMMENTS:

Interval from module preparation to inoculation - 5 days.

THERMAL VACUUM ACCEPTANCE TEST
#301 Hardware

July 31 - August 9, 1966

Heterokaryotic survival from plating data

Test Position	Conidia exposed to 5000 R before test		Unirradiated conidia	
	Proportion of all conidia	% of original Heterokaryotic fraction (0.2105)	Proportion of all conidia	% of original Heterokaryotic fraction (0.2105)
A 809	.0766	36.39	.1318	62.61
A 816	.0766	36.39	.1133	53.82
A 817	.1054	50.07	.1452	68.98
A 818	.0843	40.05	.1436	68.22
A 819	.0789	37.48	.1164	55.30
Average of experimental conidia	<u>.0844</u>	<u>40.10</u>	<u>.1301</u>	<u>61.81</u>
Control (room temp.)	.0724	34.39	.1835	37.17
Control (refrig.)	.0938	44.56	.1736	82.47
Control average	<u>.0831</u>	<u>39.48</u>	<u>.1786</u>	<u>84.85</u>

THERMAL VACUUM ACCEPTANCE TEST
#301 Hardware

July 31 - August 9, 1966

Heterokaryotic survival and mutation data from individual jugs

Test Position	Treatment	Mutation frequency X 10 ⁶		Heterokaryotic survival		
		Individual jugs	Mean	Individual jugs	Mean	Percent of original Heterokaryotic fraction (0.2105)
A 809	5000 R before test	111.7 131.0 102.9	115.2	.0888 .0683 .0857	.0809	38.43
	Unirradiated	0 0 1.0	0.3	.1726 .1560 .1673	.1653	78.53
A 816	5000 R before test	146.0 114.8 139.3	133.3	.0784 .0855 .0814	.0818	38.86
	Unirradiated	1.3 1.2 1.1	1.2	.1131 .1236 .1315	.1227	58.29
A 817	5000 R before test	123.0	123.0	.0918	.0918	43.61
	Unirradiated	0 0 1.1	0.4	.1181 .1356 .1245	.1261	59.90
A 818	5000 R before test	131.0 160.7 163.5	151.7	.0896 .0731 .0841	.0823	39.10
	Unirradiated	1.7 0 0	0.6	.1633 .1593 .1428	.1551	73.68
A 819	5000 R before test	102.6 151.9 137.8	130.8	.0743 .1158 .0729	.0893	42.42
A 819	Unirradiated	1.1 2.4 3.8	2.4	.1262 .1170 .1074	.1168	55.49
Control (room temp.)	5000 R before test	135.3 128.7 154.5	139.5	.0839 .0961 .0908	.0903	42.90
	Unirradiated	0.9 0 0.9	0.6	.1789 .1883 .1839	.1837	87.27
Control (refrig.)	5000 R before test	124.2 131.5 99.2	118.3	.0994 .0947 .0804	.0915	43.47
	Unirradiated	0.8 0 0	0.3	.1839 .1624 .1998	.1820	86.46
Mean mutation frequency for irradiated conidia in T/U chamber		132.0 x 10 ⁻⁶				
Mean mutation frequency for irradiated control conidia		128.9 x 10 ⁻⁶				
Mean heterokaryotic survival for irradiated conidia in T/u chamber				.0842	40.00	
Mean heterokaryotic survival for irradiated control conidia				.0909	43.18	
Mean heterokaryotic survival for unirradiated conidia in T/U chamber				.1372	65.18	
Mean heterokaryotic survival for unirradiated control conidia				.1829	86.89	

VIBRATION ACCEPTANCE TEST
#302 Hardware

August 26 - September 2, 1966

Heterokaryotic survival from plating data

Test Position	Conidia exposed to 5000 R before test		Unirradiated conidia	
	Proportion of all conidia	% of original Heterokaryotic fraction (0.2000)	Proportion of all conidia	% of original Heterokaryotic fraction (0.2000)
A 809	.1225	61.25	.1739	86.95
A 816	.1150	57.50	.1745	87.25
A 817	.1242	62.10	.1576	78.80
A 818	.1104	55.20	.1874	93.70
A 819	.0991	49.50	.1851	92.50
Average of experimental conidia	<u>.1142</u>	<u>57.10</u>	<u>.1757</u>	<u>87.85</u>
Control (room temp.)	.1240	62.00	.1711	85.55
Control (refrig.)	.1016	50.80	.1773	88.65
Control (room temp., no housing)	.1117	55.85	.1751	87.55
Average of controls	<u>.1124</u>	<u>56.20</u>	<u>.1745</u>	<u>87.25</u>

VIBRATION ACCEPTANCE TEST
#302 Hardware

June 30 - July 5, 1966

Heterokaryotic survival and mutation data for individual jugs

Test Position	Treatment	Mutation frequency X 10 ⁶		Heterokaryotic survival		
		Individual jugs	Mean	Individual jugs	Mean	Percent of original Heterokaryotic fraction (0.2000)
A 809	5000 R x-rays before	119.8 92.8	106.3	.1166 .1205	.1186	59.30
	Unirradiated	0 0 0	0	.1987 .1752 .2026	.1922	96.10
A 816	5000 R x-rays before	102.7 90.2 99.1	97.3	.0830 .1194 .1178	.1067	53.35
	Unirradiated	0.6 0 0	0.2	.2064 .2010 .1942	.2005	100.25
A 817	5000 R x-rays before	64.4 94.0 134.2	97.5	.1274 .1379 .0955	.1203	60.15
	Unirradiated	0 0 0	0	.1418 .1483 .1551	.1484	74.20
A 818	5000 R x-rays before	94.9 102.0 124.7	107.2	.1060 .1207 .1030	.1099	54.95
	Unirradiated	0.7 0	0.4	.1734 .1696	.1715	85.75
A 819	5000 R x-rays before	98.4 132.8	.1156	.0956 .0958	.0957	47.85
A 819	Unirradiated	0 0.7 0.8	0.5	.1887 .1831 .1675	.1798	89.90
Control (room temp.-housing)	5000 R x-rays before	111.2 117.2	114.2	.1085 .1209	.1147	57.35
	Unirradiated	0 0 0	0	.1601 .1747 .1211	.1520	76.00
Control (room temp.-no housing)	5000 R x-rays before	191.8 206.8 128.7	175.8	.0634 .0667 .0882	.0728	36.40
	Unirradiated	0 0.8	0.4	.1744 .1478	.1611	80.55
Mean mutation frequency for irradiated conidia (Vibrated)			103.8			
Mean mutation frequency for irradiated unvibrated conidia			151.1			
Mean heterokaryotic survival for irradiated vibrated conidia				.1107	55.35	
Mean heterokaryotic survival for irradiated unvibrated conidia				.0895	44.75	
Mean heterokaryotic survival for unirradiated vibrated conidia				.1790	89.50	
Mean heterokaryotic survival for unirradiated unvibrated conidia				.1556	77.80	

SUMMARY AND COMMENTS ON VIBRATION ACCEPTANCE (302) TEST

Interval from module preparation to inoculation	7 days
Estimated average survival of unirradiated conidia - jugs	89.50%
- plates	87.85%

The 302 housings were conditioned prior to use by repeated autoclaving (3X) and washing with detergent and distilled water. This seems to have improved the survival.

THERMAL-VACUUM ACCEPTANCE TEST
#302 Hardware

September 12 - 21, 1966

Test Position	Conidia exposed to 5000 R before test		Unirradiated conidia	
	Proportion of all conidia	% of original Heterokaryotic fraction (0.1685)	Proportion of all conidia	% of original Heterokaryotic fraction (0.1685)
A 809	.1180	70.03	.1600	94.96
A 816	.1322	78.46	.1767	104.87
A 817	.1152	68.37	.1586	94.12
A 818	.1543	91.57	.1532	90.92
A 819	.1091	64.75	.1473	87.42
Average of experimental conidia	<u>.1258</u>	74.66	<u>.1592</u>	94.48
Control (room temp.-in housing)	.1140	67.66	.1776	105.40
Control (refrig.- no housing)	.1034	61.36	.1685	100.00
Average of controls	<u>.1087</u>	64.51	<u>.1731</u>	102.73

THERMAL VACUUM ACCEPTANCE TEST
#302 Hardware
September 12 - 21, 1966

Test Position	Treatment	Mutation frequency X 10 ⁶		Heterokaryotic survival		
		Individual jugs	Mean	Individual jugs	Mean	Percent of original Heterokaryotic fraction (0.1685)
A 809	5000 R x-rays before	80.5 133.4	107.0	.0947 .0889	.0918	54.48
	Unirradiated	0 0 0	0	.1359 .1596 .1465	.1473	87.42
A 816	5000 R x-rays before	-	-	-	-	-
	Unirradiated	0	0	.0849	.0849	50.39
A 817	5000 R x-rays before	146.5	146.5	.0867	.0867	51.45
	Unirradiated	0 0 0	0	.1135 .1147 .1200	.1161	68.90
A 818	5000 R x-rays before	113.5	113.5	.1189	.1189	70.56
	Unirradiated	0 0	0	.1052 .1156	.1104	65.52
A 819	5000 R x-rays before	124.5	124.5	.1087	.1087	54.51
	Unirradiated	0	0	.1002	.1002	59.47
Room temp. in housing	5000 R x-rays before	109.2	109.2	.0818	.0818	48.55
	Unirradiated	0 0	0	.1316 .1274	.1295	76.85
Refrig. no housing	5000 R x-rays before	117.5 131.8 116.2	121.8	.0772 .0932 .0781	.0828	49.14
	Unirradiated	0 0 1.2	0.4	.1370 .1285 .1208	.1288	76.44
Mean mutation rate for irradiated vibrated conidia			119.7			
Mean mutation rate for irradiated unvibrated conidia			118.7			
Mean Heterokaryotic survival for irradiated vibrated conidia				.0996		59.11
Mean Heterokaryotic survival for irradiated unvibrated conidia				.0826		49.02
Mean Heterokaryotic survival for unirradiated vibrated conidia				.1196		70.98
Mean Heterokaryotic survival for unirradiated unvibrated conidia				.1291		76.62

SUMMARY AND COMMENTS ON THERMAL VACUUM ACCEPTANCE (302) TEST

Interval from module preparation to inoculation	9 days
Estimated average survival of unirradiated conidia in test - jugs . .	70.98
- plates .	94.48

Plating shows good survival.

Jug survivals may suffer from delay in inoculation.

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