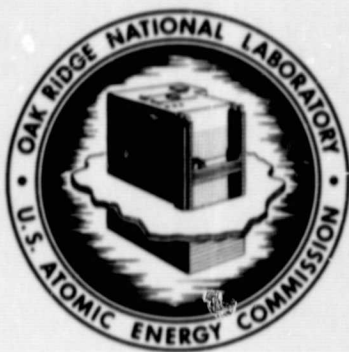


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BIOLOGY DIVISION

NEUROSPORA EXPERIMENT P-1037

FINAL REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

MARCH 1970

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OAK RIDGE NATIONAL LABORATORY  
Oak Ridge, Tennessee  
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**FINAL REPORT**  
**TO THE**  
**NATIONAL AERONAUTICS AND SPACE ADMINISTRATION**

**Title of Project:**

**Mutagenic Effectiveness of Known Doses of Radiation in Combination  
with Zero Gravity on Neurospora**

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P-1037

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THE SPACE FLIGHT RADIATION INTERACTION EXPERIMENTS WITH  
NEUROSPORA CRASSA

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## I. INTRODUCTION

Successful experiments using *Neurospora* as the test organism have been performed on two different space flights. The first experiment was in September 1966 on the Gemini XI mission and the second experiment was in September 1967 on the Biosatellite II mission. In both experiments a study was made of the genetic effects of space flight alone and space flight in combination with known doses of radiation. On the Biosatellite II mission a  $^{85}\text{Sr}$  gamma-ray source was used; on the Gemini XI mission a  $^{32}\text{P}$  beta-ray source was used. In both experiments we used a genetically marked two-component heterokaryon, heterozygous for two different genes that control sequential steps in purine biosynthesis. Mutation at either locus gives rise to a requirement for adenine as well as to accumulation of a reddish-purple pigment in the vacuoles of the mycelium. This strain was used to study radiation-induced inactivation of heterokaryotic conidia as well as radiation-induced gene mutation at two specific loci. In both experiments a range of radiation exposures were given, so that a comparison could be made between flight and ground-control dose-response curves rather than between flight and ground-control effects of some single exposure. In the Biosatellite II experiment, *Neurospora* conidia were tested while collected on the surface of Millipore filters; in the Gemini XI experiment they were tested both while collected on the surface of Millipore filters and while in a colloidal suspension of agar.



## II. RESULTS

The effects resulting from space flight on radiation-induced inactivation and the overall induction of ad-3 mutations on the Biosatellite II mission have been published (de Serres and Webber 1968). In brief, there was no difference between the flight and ground-control survival curves (Fig. 1) nor between flight and ground-control curves for the overall induction of ad-3 mutations (Fig. 2). The characterization of the ad-3 mutants recovered in this experiment is complete, and the overall induction curves have been resolved into their different components. These data (de Serres and Webber, 1970) show that there is no difference between the flight and ground-control curves for point mutations or chromosome deletions (Figs. 3—6).

The effects of space flight on radiation-induced inactivation and the overall induction of ad-3 mutations on the Gemini XI mission have also been published (de Serres et al., 1969). In brief, these data show that the space flight affected the samples flown on Millipore filters in the same way as on the Biosatellite II mission; there was no effect on survival or mutation induction (Figs. 7 and 8). Quite a different result was found with the Neurospora samples flown in suspension: the data showed that the flight samples had higher levels of survival and lower frequencies of mutation induction (Figs. 7 and 8). Space flight gave protection against both effects of radiation on samples in suspension.

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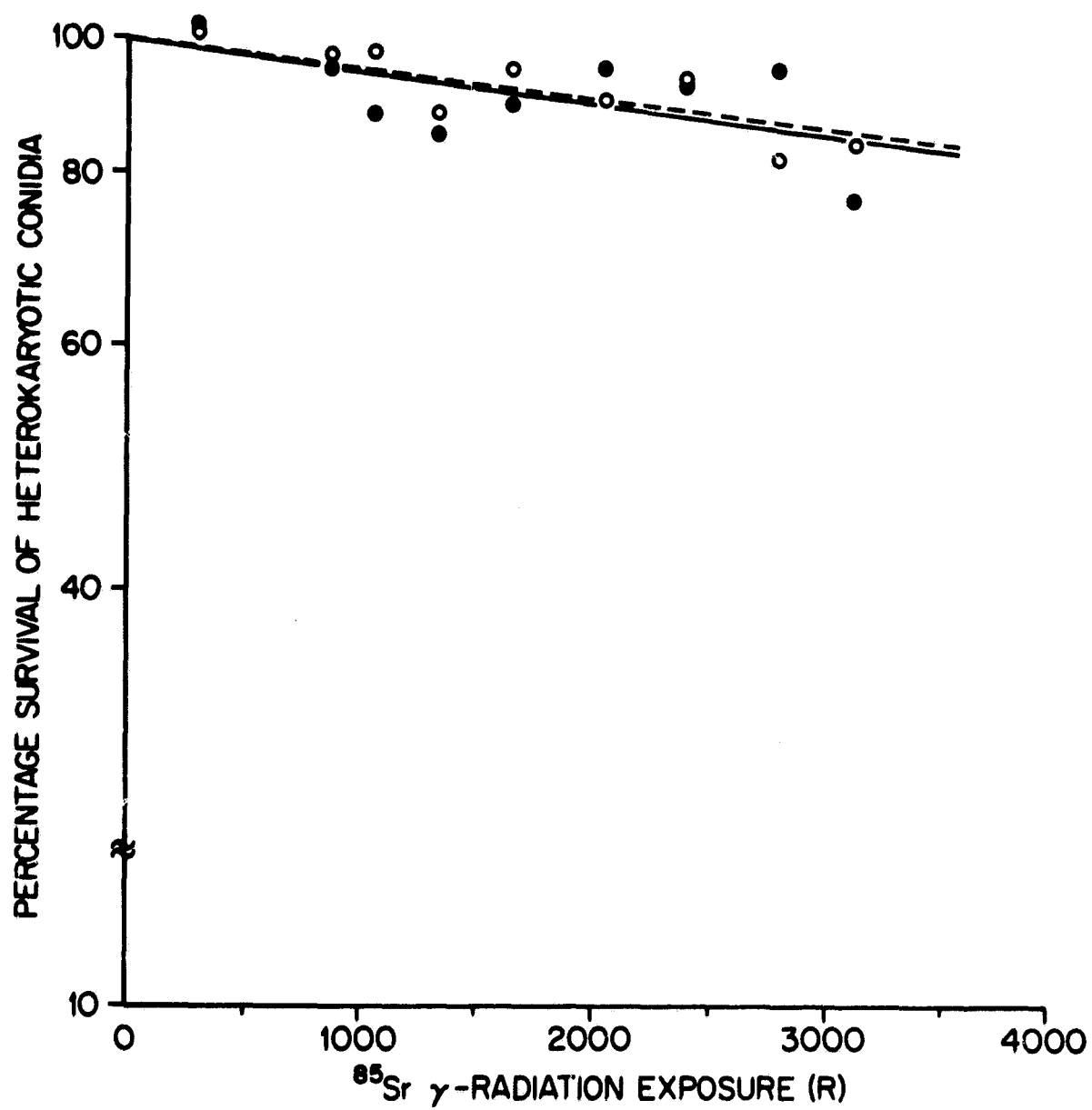


Fig. 1. Dose-Effect Curves for Survival on Biosatellite II. (—, ● = Flight samples; --, ○ = ground-control samples.)

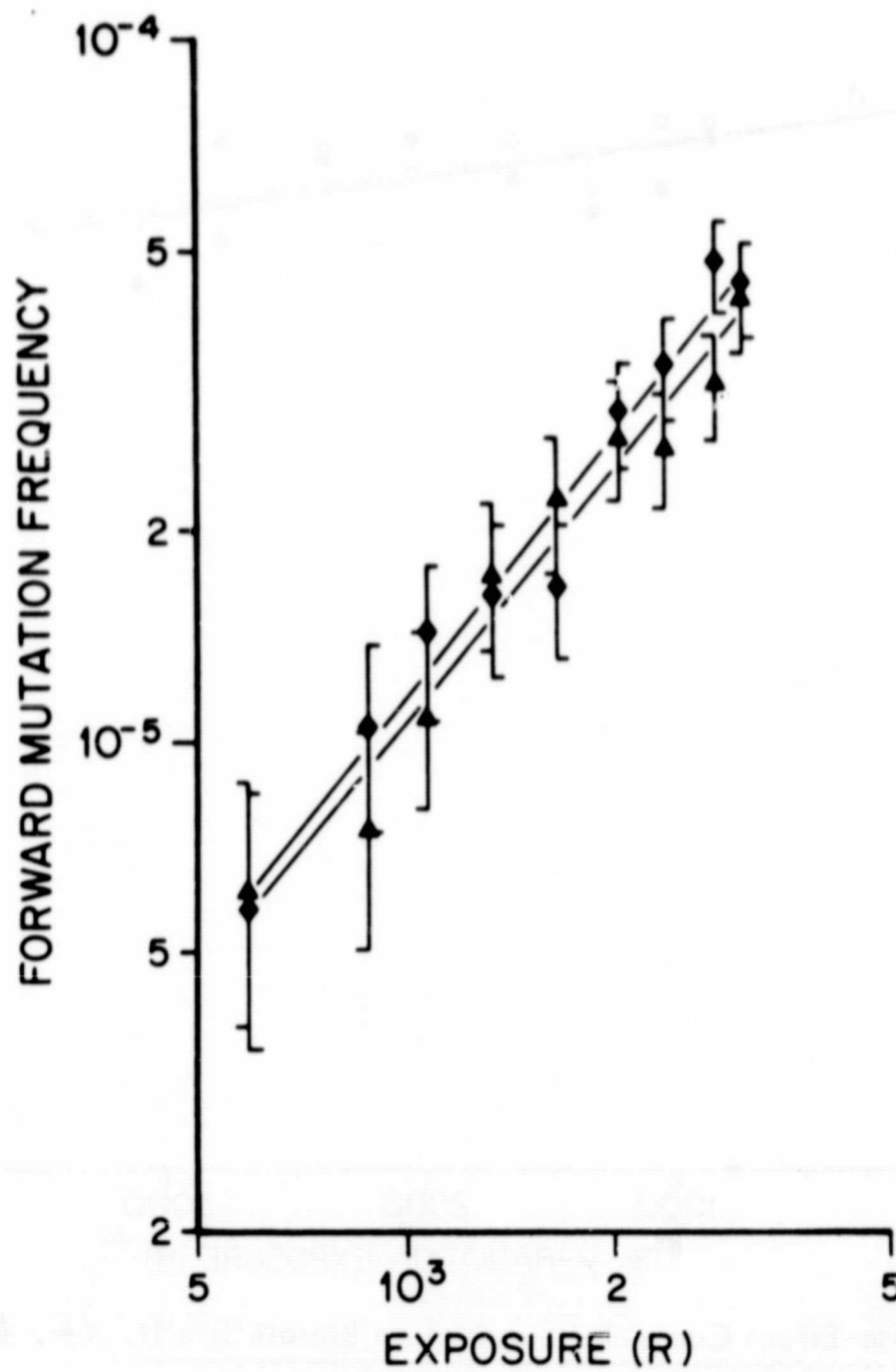


Fig. 2. Comparison of Overall Induction Curves for Recessive Lethal Mutations in the ad-3 Region on Biosatellite II.

(▲ = Flight samples; ◆ = ground-control samples.)

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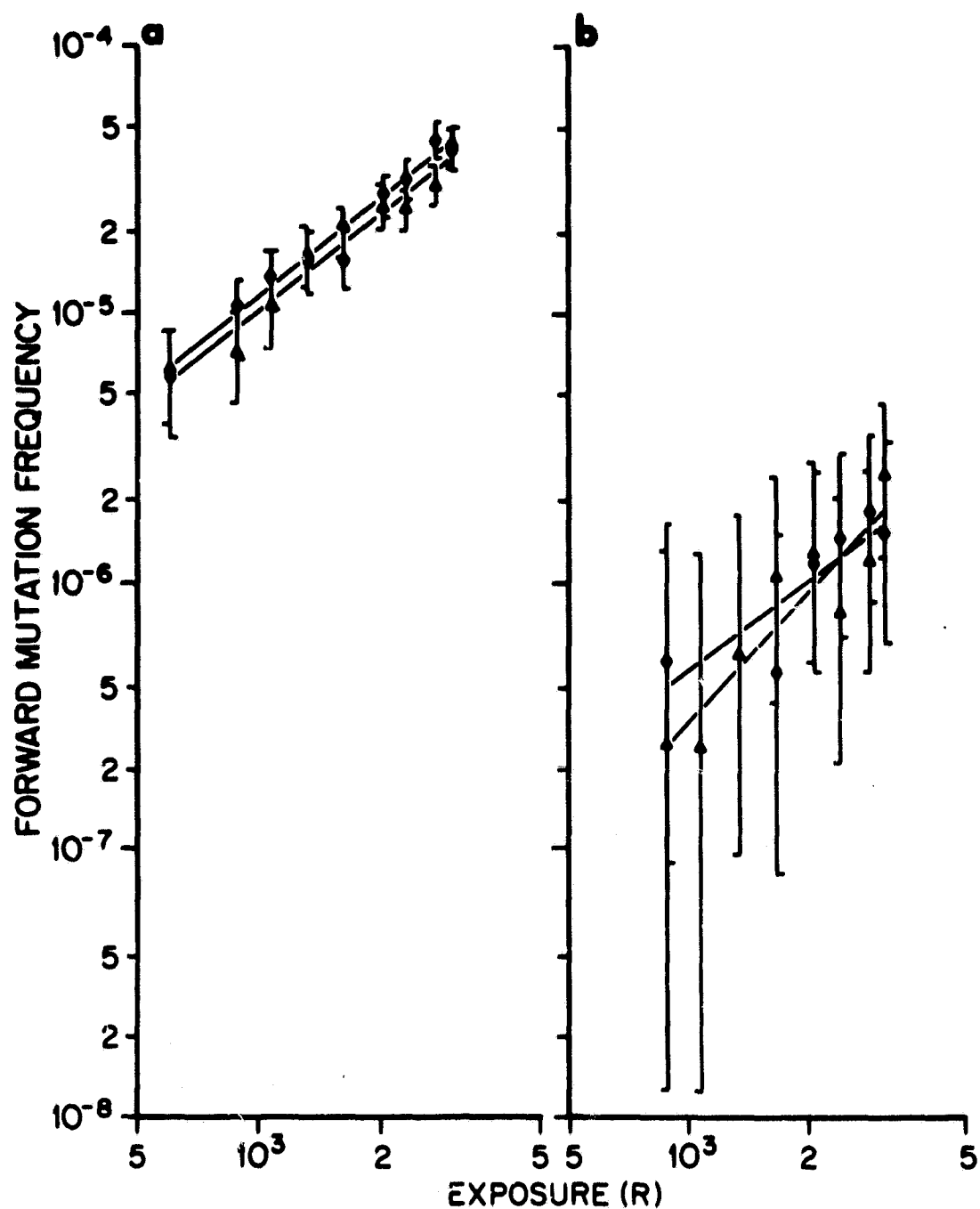


Fig. 3. Comparisons of Induction Curves on Biosatellite II.

(a)  $ad-3^R$  mutations, (b)  $ad-3^{IR}$  mutations. (▲ = Flight samples;  
◆ = ground-control samples.)

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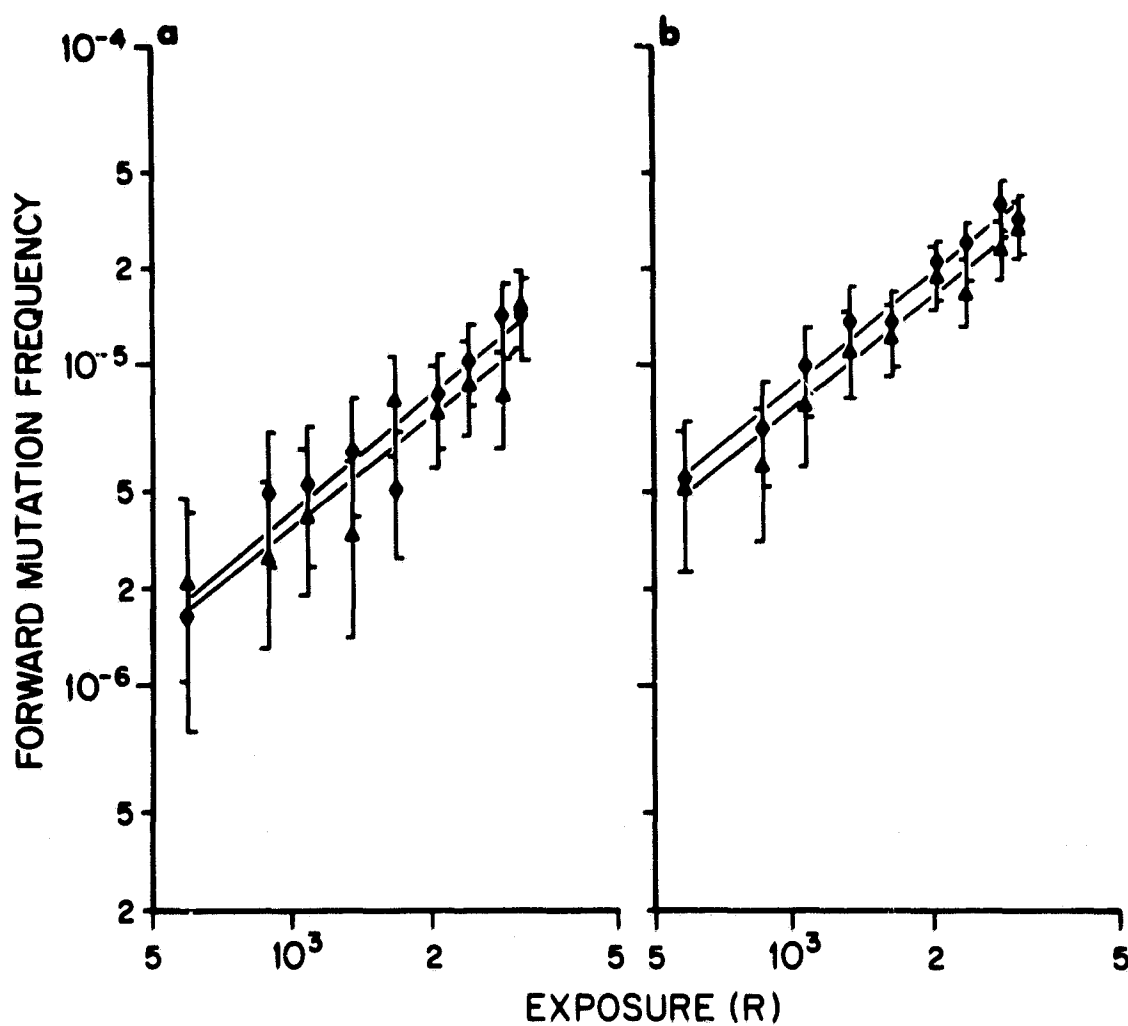


Fig. 4. Comparisons of Induction Curves on Biosatellite II. (a)  $\text{ad-3A}^R$  mutations, (b)  $\text{ad-3B}^R$  mutations. ( $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

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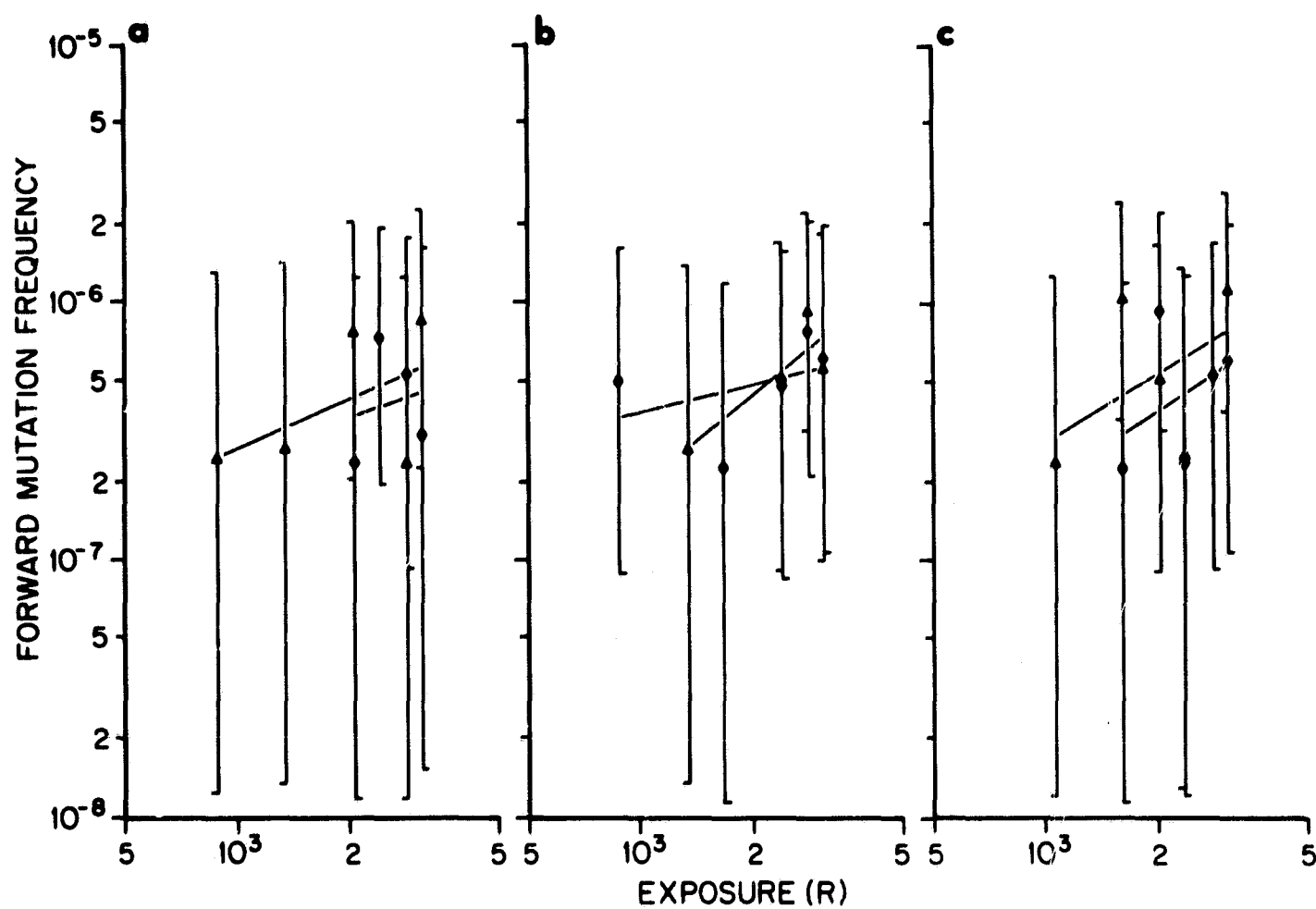


Fig. 5. Comparisons of Induction Curves on Biosatellite II.

(a)  $\text{ad-3A}^{\text{IR}}$  mutations, (b)  $\text{ad-3B}^{\text{IR}}$  mutations, (c)

$(\text{ad-3A ad-3B})^{\text{IR}}$  mutations. (  $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

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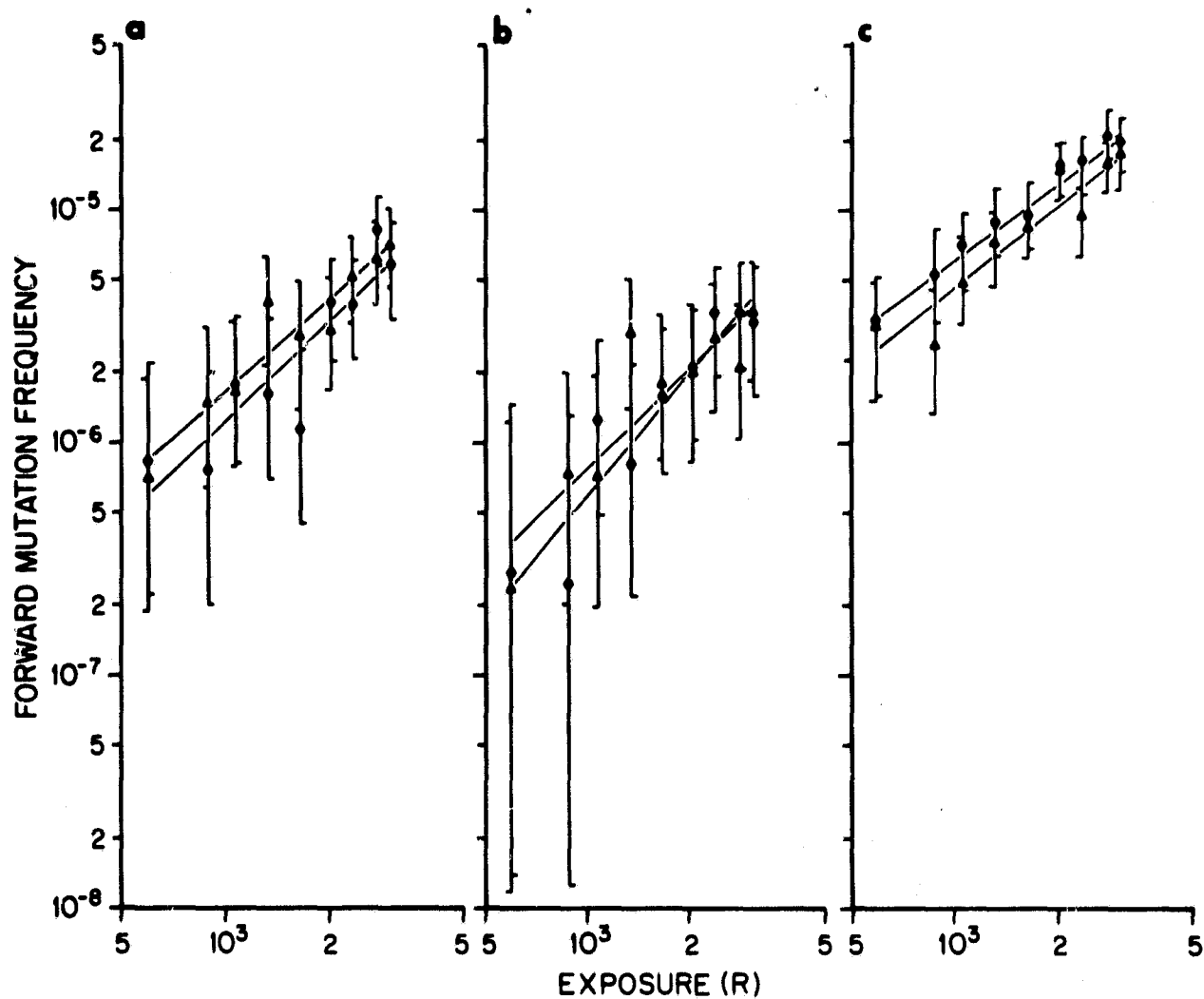


Fig. 6. Comparisons of Induction Curves on Biosatellite II.

(a)  $\text{ad-3B}^{\text{R-NP}}$  mutations, (b)  $\text{ad-3B}^{\text{R-P}}$  mutations,

(c)  $\text{ad-3B}^{\text{R-NC}}$  mutations. ( $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

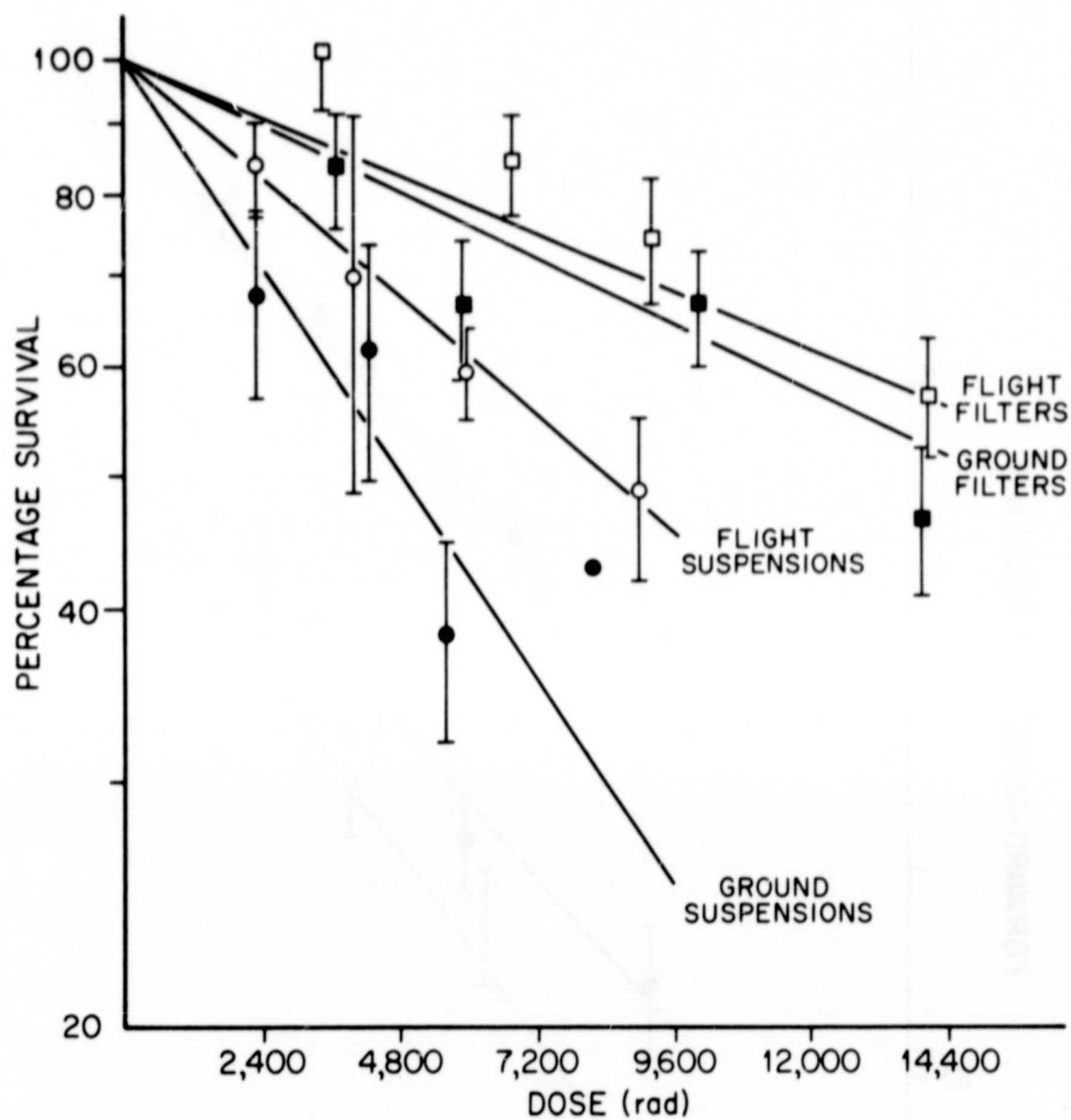


Fig. 7. Survival after  $^{32}\text{P}$  Irradiation on Gemini XI. Conidia were collected on Millipore filters or prepared as suspensions for both flight and ground-control. (  $\square$  = Flight filter samples;  $\blacksquare$  = ground filter samples;  $\circ$  = flight suspension samples;  $\bullet$  = ground suspension samples.)



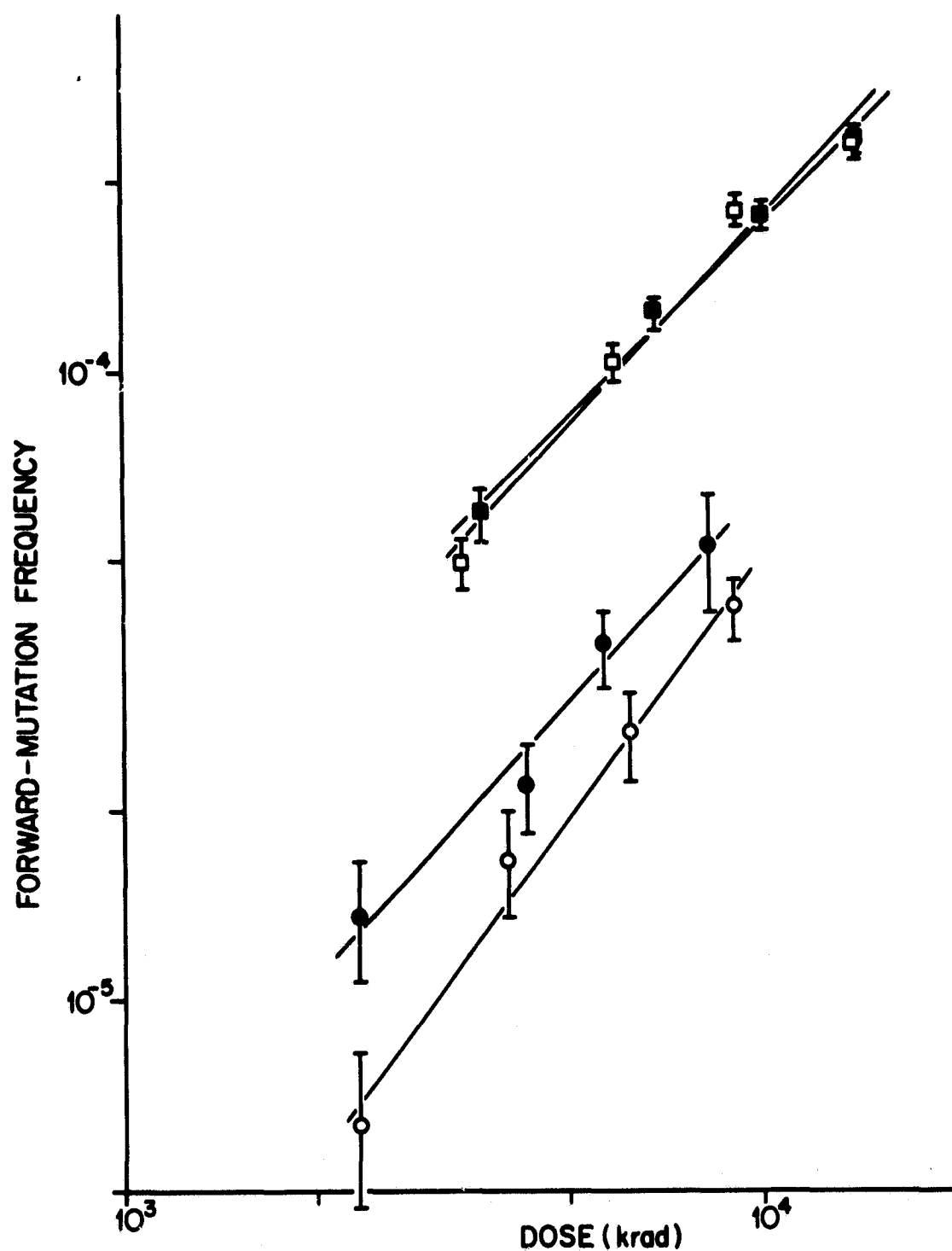


Fig. 8. Forward-Mutation Frequencies for Recessive Lethal Mutations in the ad-3 Region after <sup>32</sup>P Irradiation on Gemini XI. Conidia were collected on Millipore filters or prepared as suspensions for both flight and ground control. ( □ = Flight filter samples; ■ = ground filter samples; ○ = flight suspension samples; ● = ground suspension samples.)

Genetic analysis of the ad-3 mutants recovered in the Gemini XI experiment is complete, and the overall induction curves have been resolved into their individual components. These data (de Serres and Smith, 1970) show that the genetic analysis of the ad-3 mutants in the filter samples gave the same results in the Gemini XI experiment as in the Biosatellite II experiment. No difference was found between the flight and ground-control curves for point mutations or chromosome deletions (Figs. 9—12).

The genetic analysis of the ad-3 mutants recovered from the samples in suspension gave quite different results. These data show that the lower frequencies of mutation induction in the flight samples was due to a specific effect on point mutations and not on chromosome-deletion mutations (Figs. 13—16).

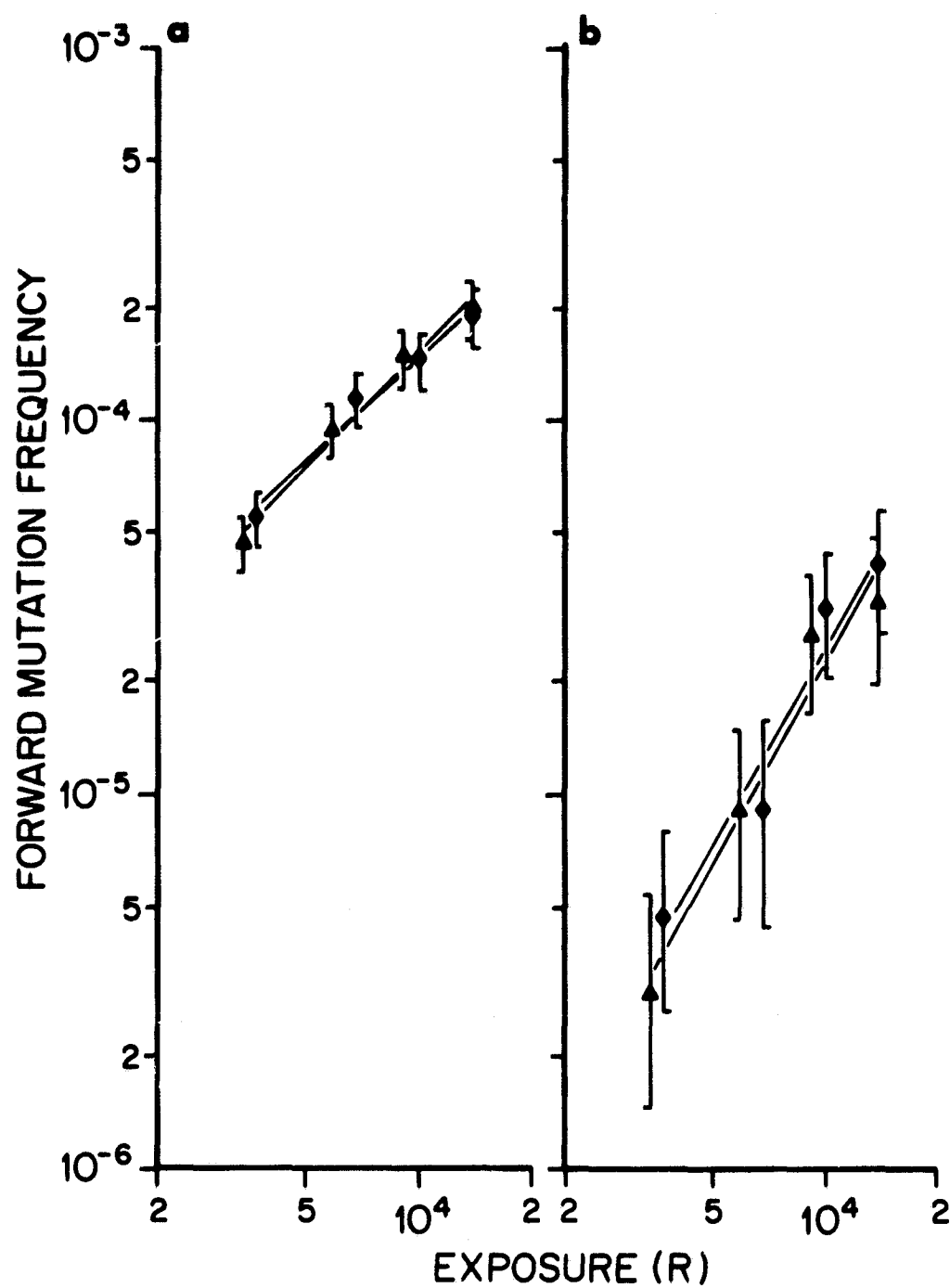


Fig. 9. Forward-Mutation Frequencies for Spore Samples on Millipore Filters in the Gemini XI Experiment. (a)  $ad-3^R$  mutations, (b)  $ad-3^{IR}$  mutations. (  $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

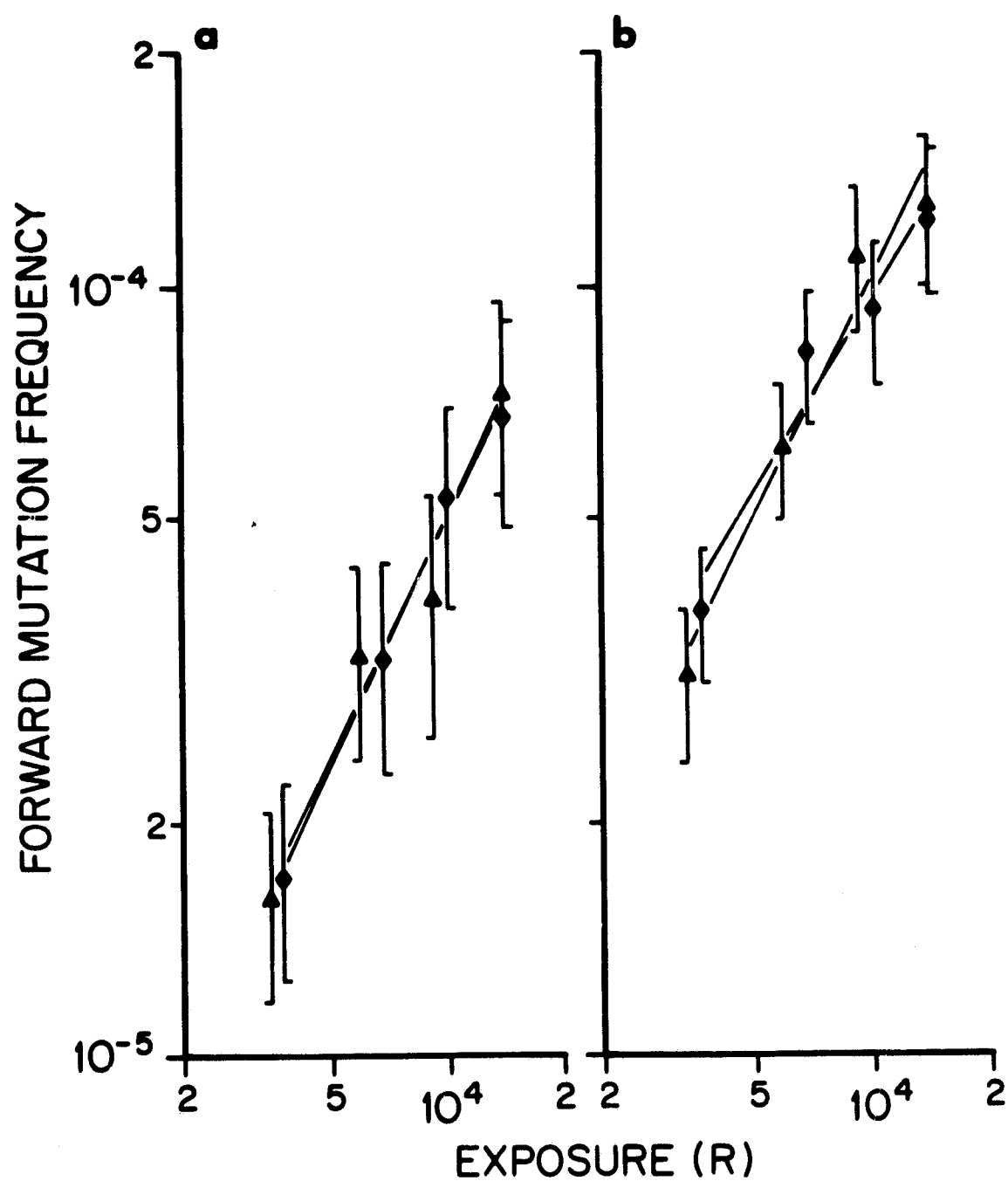


Fig. 10. Forward-Mutation Frequencies for Spore Samples on Millipore Filters in the Gemini XI Experiment. (a)  $ad-3A^R$  mutations, (b)  $ad-3B^R$  mutations. ( $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

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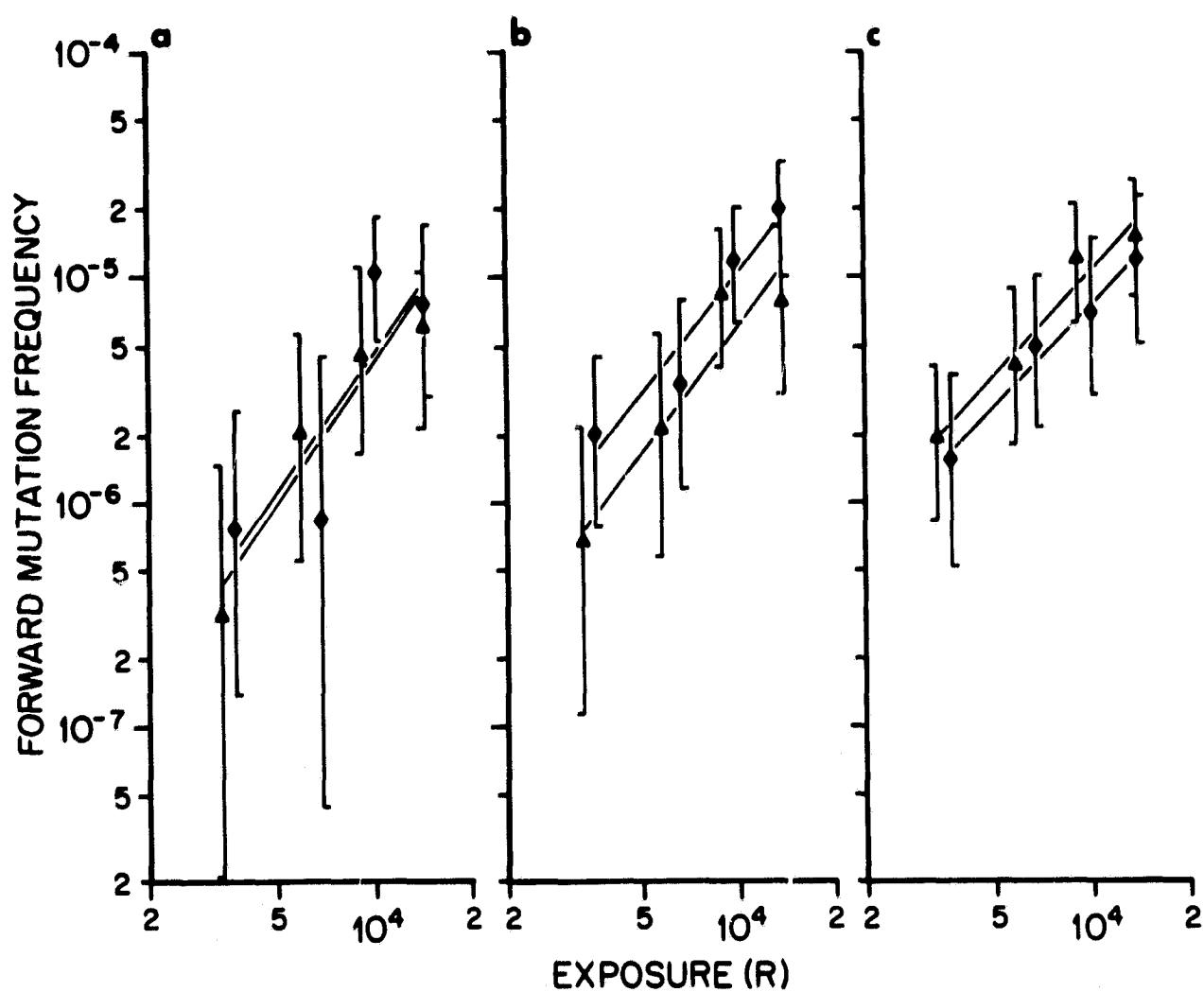


Fig. 11. Forward-Mutation Frequencies for Spore Samples on Millipore Filters in the Gemini XI Experiment. (a)  $ad-3A^{IR}$  mutations, (b)  $ad-3B^{IR}$  mutations, (c)  $(ad-3A ad-3B)^{IR}$  mutations. ( $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

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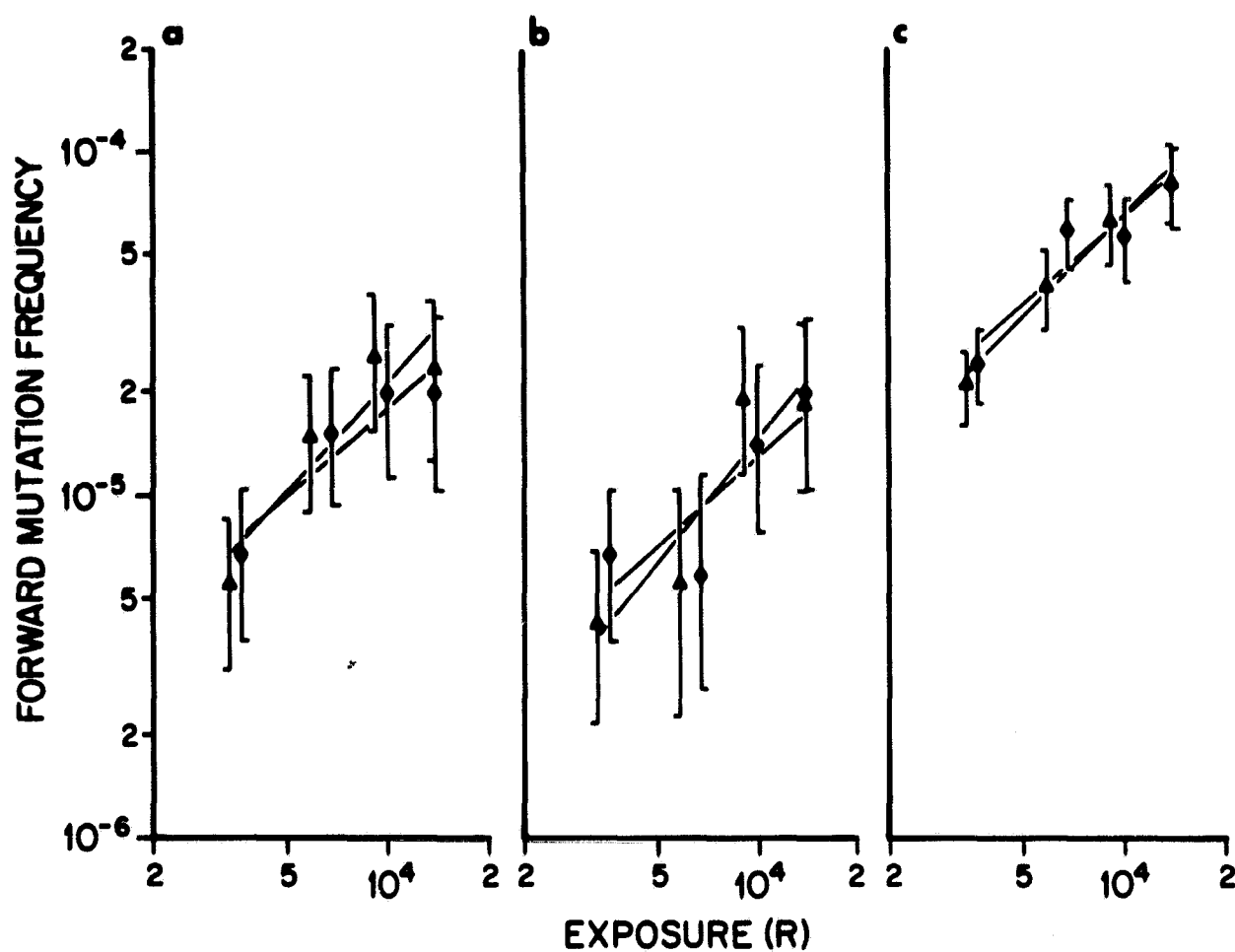


Fig. 12. Forward-Mutation Frequencies of  $\text{ad-3B}^{\text{R}}$  Mutants as a Function of Complementation Pattern for Spore Samples on Millipore Filters in the Gemini XI Experiment. (a) Nonpolarized— $\text{ad-3B}^{\text{R-NP}}$  mutations, (b) polarized— $\text{ad-3B}^{\text{R-P}}$  mutations, (c) noncomplementing— $\text{ad-3B}^{\text{R-NC}}$  mutations. ( $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

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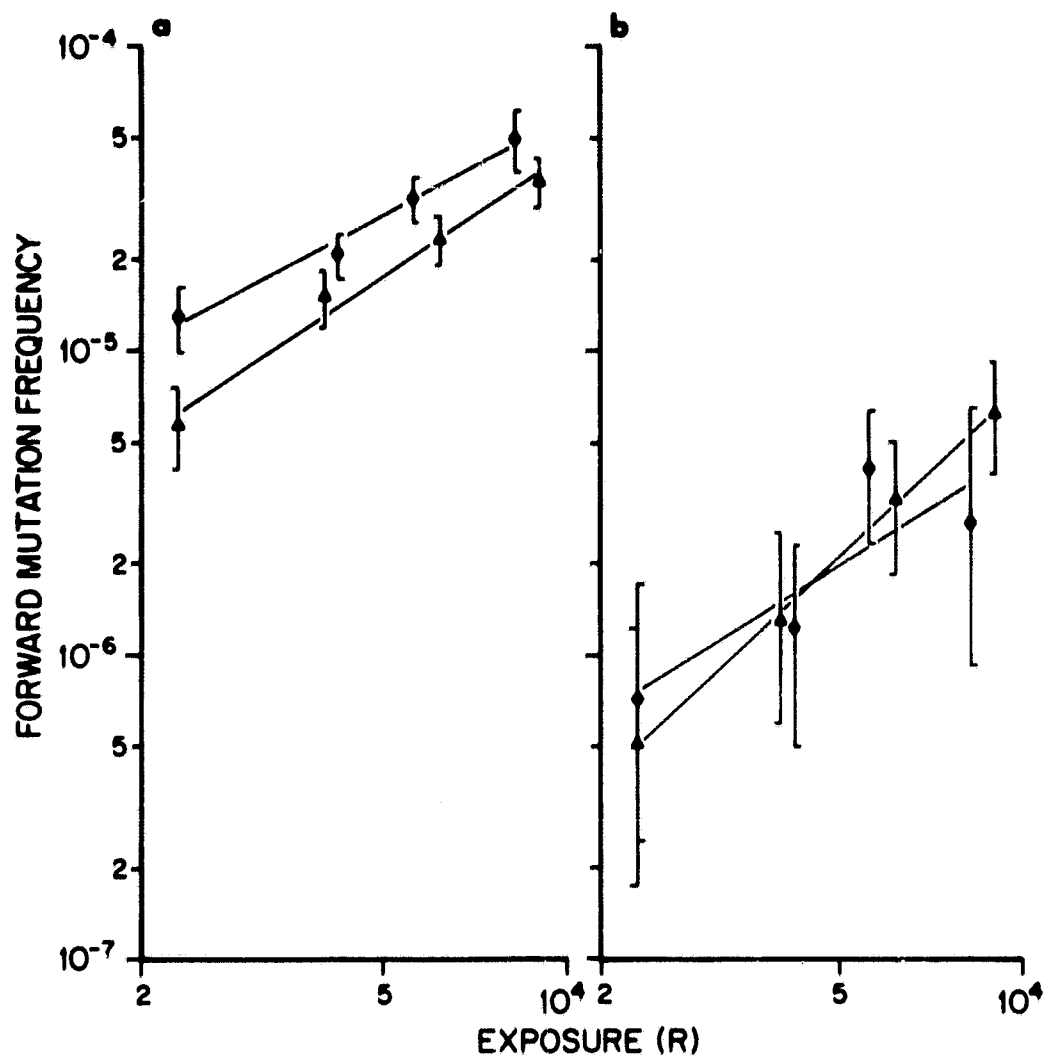


Fig. 13. Forward-Mutation Frequencies for Spore Samples in Suspension on Gemini XI. (a)  $ad-3^R$  mutations, (b)  $ad-3^{IR}$  mutations. ( $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

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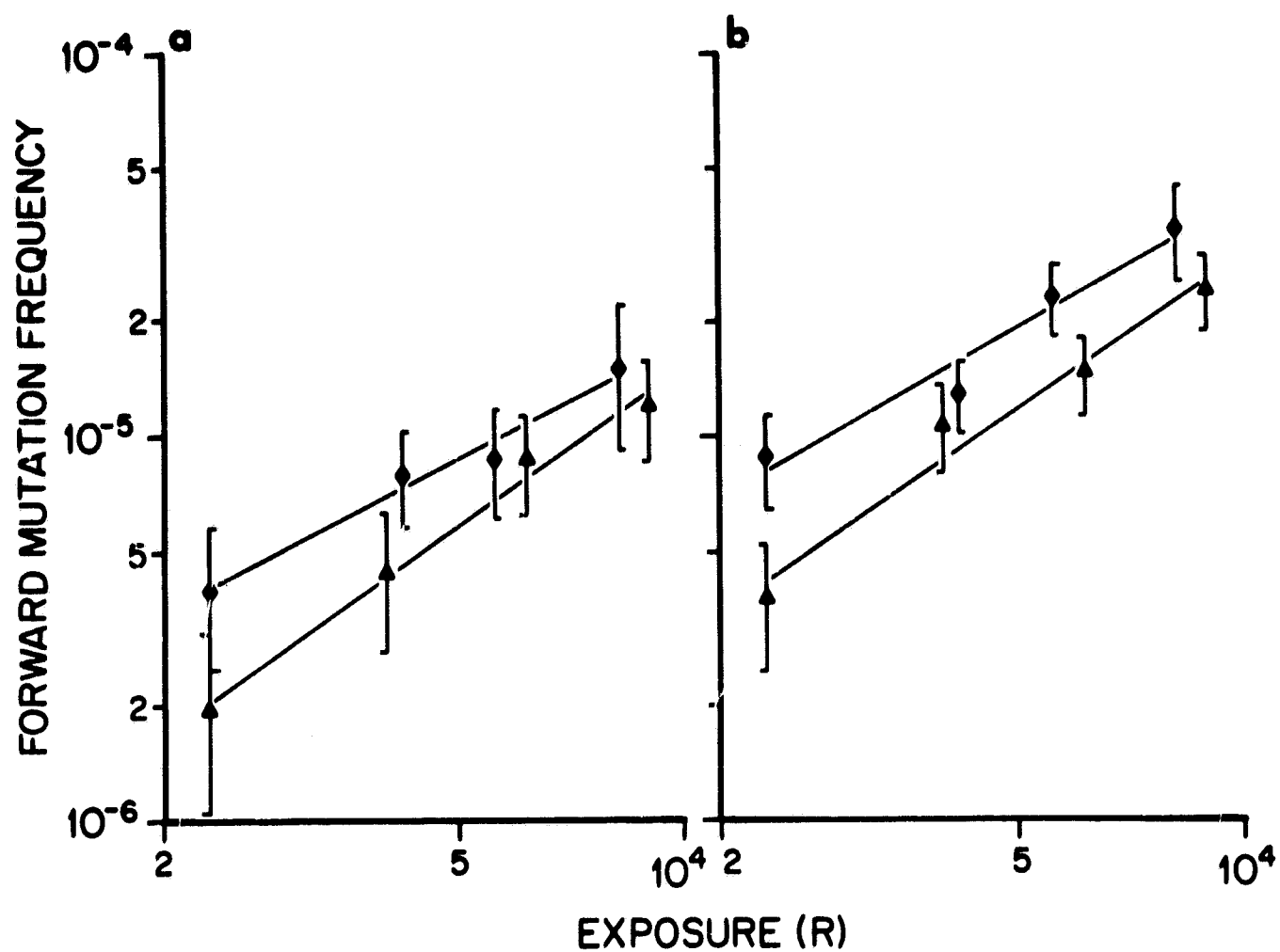


Fig. 14. Forward-Mutation Frequencies for Spore Samples in Suspension on Gemini XI. (a)  $ad-3A^R$  mutations, (b)  $ad-3B^R$  mutations. (▲ = Flight samples; ◆ = ground-control samples.)



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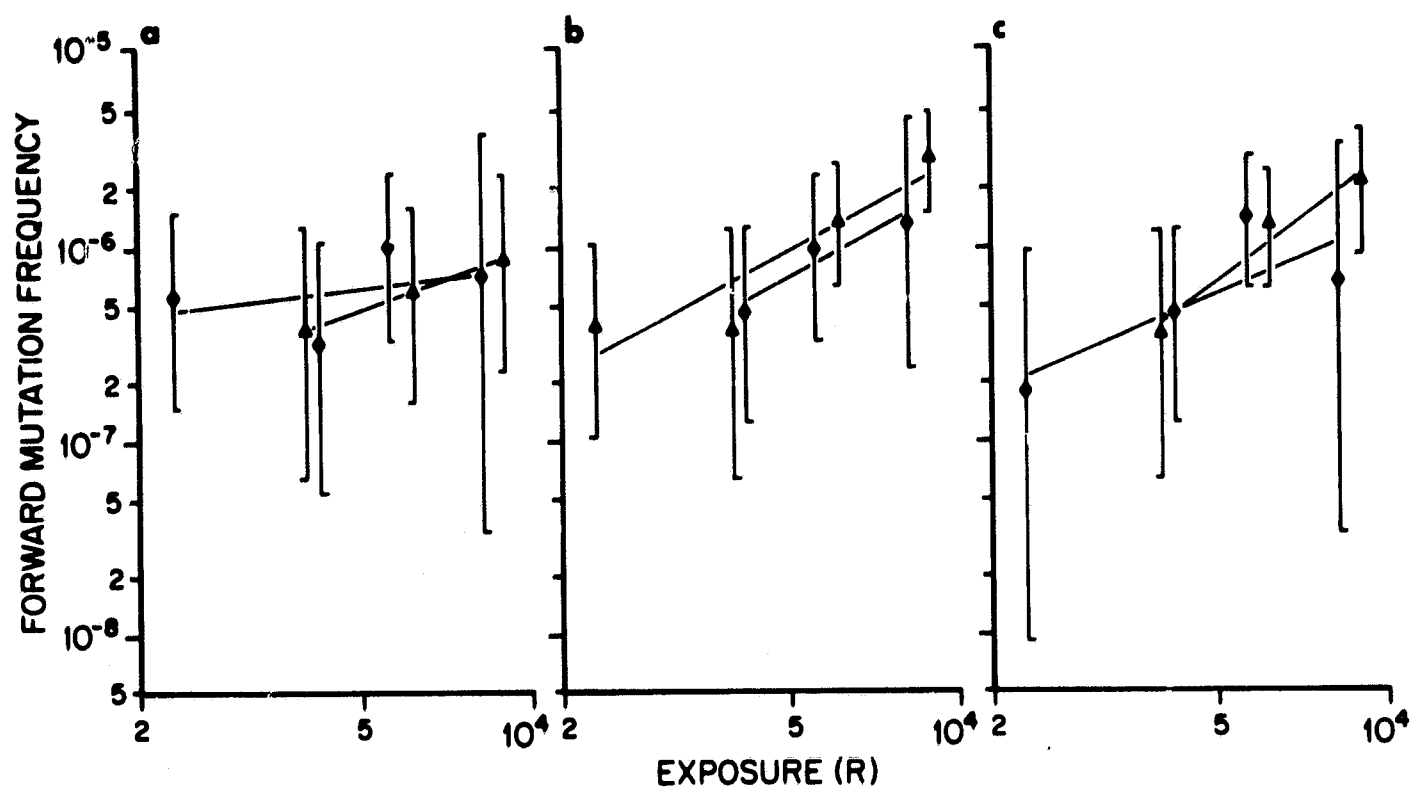


Fig. 15. Forward-Mutation Frequencies for Spore Samples in Suspension on Gemini XI. (a)  $ad-3A^{IR}$  mutations, (b)  $ad-3B^{IR}$  mutations, (c)  $(ad-3A ad-3B)^{IR}$  mutations. (  $\blacktriangle$  = Flight samples;  $\blacklozenge$  = ground-control samples.)

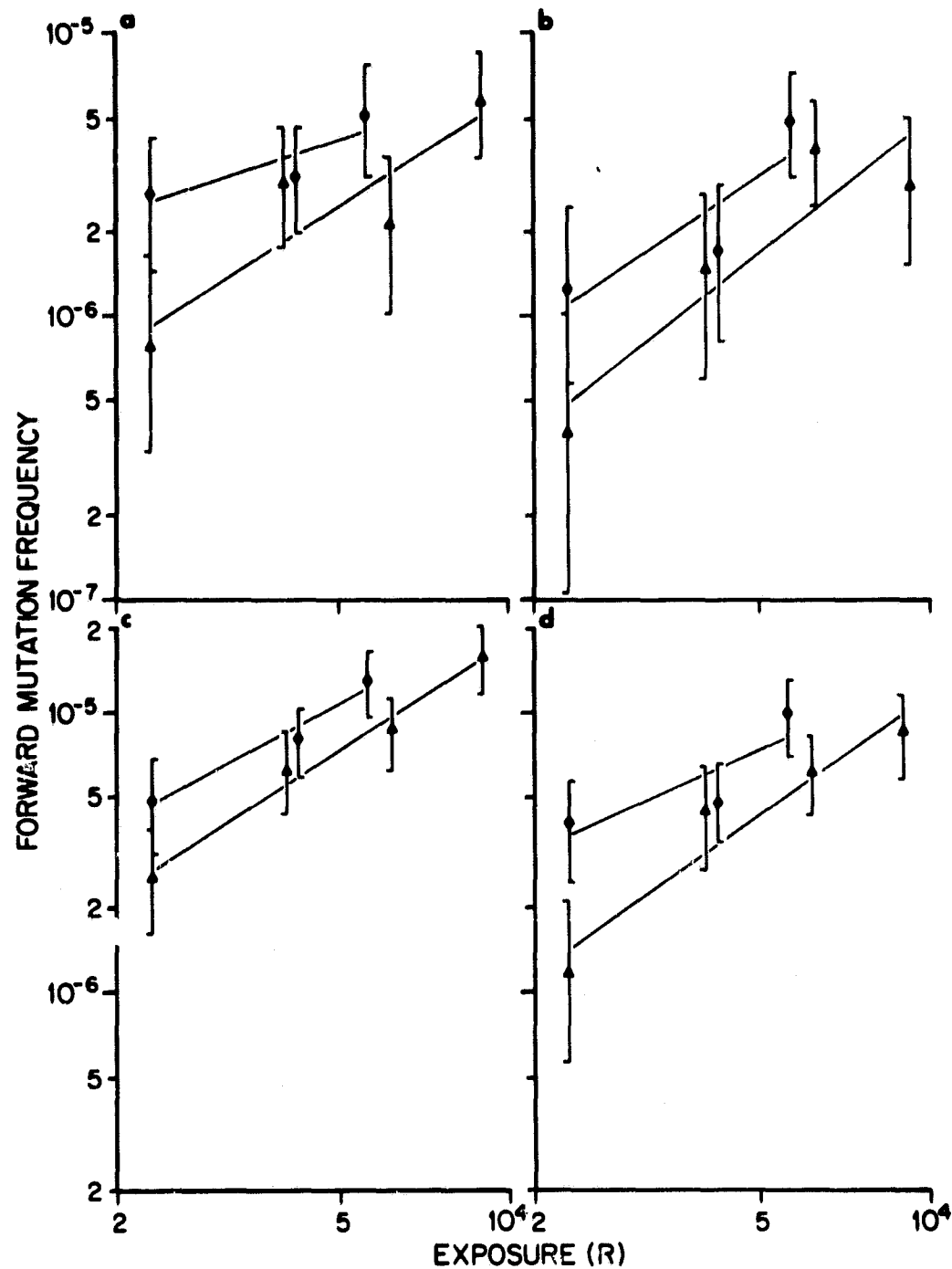


Fig. 16. Forward-Mutation Frequencies of  $\text{ad-3B}^{\text{R}}$  Mutations as a Function of Complementation Pattern for Spore Samples in Suspension on Gemini XI. (a) Nonpolarized— $\text{ad-3B}^{\text{R-NP}}$ , (b) polarized— $\text{ad-3B}^{\text{R-P}}$ , (c) noncomplementing— $\text{ad-3B}^{\text{R-NC}}$ , (d) total complementing— $\text{ad-3B}^{\text{R-C}}$ . (▲ = Flight samples; ◆ = ground-control samples.)

### III. DISCUSSION

The possibility was considered that the higher levels of survival and the lower forward-mutation frequencies might be due to anoxia because of a difference in the temperature profiles of the spacecraft cabin and the ground-control chamber prior to irradiation. So an experiment was performed to study the effects of anoxia on radiation-induced inactivation and induction of mutations.

In contrast to our Gemini XI data, we found that anoxia gives a reduction in the frequencies of both point mutations and chromosome-deletion mutations. In addition, there is a marked difference between the two spectra of point mutations as well as those of chromosome-deletion mutations. With anoxia the effect on chromosome deletions is correlated with size:  $(\underline{\text{ad-3A ad-3B}})^{\text{IR}} > \underline{\text{ad-3B}}^{\text{IR}} > \underline{\text{ad-3A}}^{\text{IR}}$ . Tests for allelic complementation showed that with anoxia there was a different spectrum of complementation patterns: whereas there was no effect of anoxia on the frequency of mutants with polarized patterns, there was a 2.7-fold reduction in the frequency of mutants with nonpolarized patterns and noncomplementing mutants.

The fundamental difference between the two Neurospora experiments on the Gemini XI mission is that the conidia on Millipore filters are essentially metabolically inactive, whereas the spores in suspension are metabolically very active. The various experiments on the Biosatellite II mission have shown that the effects of weightlessness on radiation-induced genetic damage are complex;

both antagonistic and synergistic effects of radiation have been found. The result depends on the assay system. In most cases, however, the effects are small, 2- to 5-fold differences being the usual order of magnitude.

It is important to remember that these experiments were intended as survey experiments to determine whether there were genetic mechanisms on which space flight might have an effect. It is only with the proper followup experimentation that we can hope to determine whether the effects that we have found are due to weightlessness and whether 2- to 5-fold differences are the maximum level of these effects.

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