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QUARTERLY PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

April 1 - June 30, 1968

F. J. de Serres
B. B. Webber

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BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

April 1 - June 30, 1968

JUNE 1969

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QUARTERLY PROGRESS REPORT
TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Title of Project:

Mutagenic Effectiveness of Known Doses of Gamma Irradiation in Combination with Zero Gravity on Neurospora

For the Period:

April 1 - June 30, 1968

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Interagency Agreement:

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Support:

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ABSTRACT

The Neurospora experiment on the Biosatellite-II mission utilized a two-component heterokaryon to study the effect of weightlessness on the production of radiation-induced recessive lethal mutations at two specific loci in the ad-3 region. The overall experiment consists of the genetic analysis of samples flown on the Biosatellite-II mission as well as those used in a series of ground-based control experiments. The analysis of this material is incomplete; this report summarizes present data and discusses their implications with regard to the effect of weightlessness on the modification of radiation-induced genetic effects. In brief, present data show that there is little or no effect of weightlessness on the production of radiation-induced recessive lethal mutations in metabolically inactive spores.

THE EFFECT OF WEIGHTLESSNESS ON THE INDUCTION OF RECESSIVE LETHAL MUTATIONS IN THE NEUROSPORA EXPERIMENT ON THE BIOSATELLITE II MISSION

F. J. de Serres B. B. Webber

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I. INTRODUCTION

The Neurospora experiment on the Biosatellite II mission was designed to detect any effect of weightlessness on the production of radiation-induced recessive lethal mutations. The development of a two-component heterokaryon carrying two different haploid nuclei, each marked with a series of biochemical markers, has made it possible to study diploid effects with a haploid organism (de Serres and Osterbind, 1962). In this heterokaryon (referred to as a dikaryon) recessive lethal mutations occurring at two specific loci in the ad-3 region, ad-3A and ad-3B, result both from point mutation and chromosome deletion.

The Neurospora assay system was used to investigate the genetic effects of weightlessness on the Gemini XI mission (de Serres et al., 1969; de Serres and Smith, 1969). In that experiment it was possible to assay for an effect of weightlessness with spores in suspension and with spores collected on the surface of Millipore filters. The Biosatellite II mission made it possible to confirm the results obtained with spores on Millipore filters on the Gemini XI mission.

Analysis of the Biosatellite II and postflight control experimental material is incomplete. The purpose of this report is to summarize present data and to discuss their implications with regard to the effect of weightlessness on the modification of radiation-induced genetic effects.

II. EXPERIMENTAL DESIGN

The primary objective of this study is to determine the effect of weightlessness on the frequency of radiation-induced recessive lethal mutations at two specific loci in the ad-3 region of Neurospora. The experiment utilizes metabolically inactive asexual spores collected on the surface of Millipore filters. The genetic composition of the two-component heterokaryon and a description of the Neurospora experiment package, the passive dosimetry systems, etc. have been presented previously (de Serres and Webber, 1968).

Primary data consist of the overall curve for the induction of ad-3 mutations. These mutations are characterized by a series of genetic tests (de Serres and Smith, 1969) to resolve this curve into its different components. The overall curve has two main components, $ad\text{-}3^{R}$ mutations (point mutations) and $ad\text{-}3^{IR}$ mutations (chromosome deletions), both of which can be resolved into smaller components: $ad\text{-}3^{R}$ mutations consist of point mutations at the ad-3A locus, $ad\text{-}3A^{R}$, and at the ad-3B locus, $ad\text{-}3B^{R}$; $ad\text{-}3^{IR}$ mutations consist of multilocus deletion mutations that inactivate either the ad-3A or ad-3B locus, or both simultaneously: $ad\text{-}3A^{IR}$, $ad\text{-}3B^{IR}$ and $(ad\text{-}3A\ ad\text{-}3B)^{IR}$. Point mutations at the ad-3B locus may be classified according to complementation pattern: nonpolarized, $ad\text{-}3B^{R\text{-}NP}$; polarized $ad\text{-}3B^{R\text{-}P}$ and noncomplementing, $ad\text{-}3B^{R\text{-}NC}$.

III. COMPARISONS BETWEEN FLIGHT DATA AND GROUND-CONTROL DATA

The Neurospora experiment consists of (1) a preflight ground control experiment, Phase A (the ground control portion of the unsuccessful flight of Biosatellite A in Dec. 1966); (2) flight and simultaneous ground control experiments (Biosatellite II); and (3) two different postflight ground control experiments both without (Phase B) and with hard environments (Phase C). The experimental design numbers assigned to each portion of these experiments are given in Table 1. Ground control experiments performed in 201 hardware in the 201 capsule are numbered 59-2, 73-2, 89-2, and 94-2. Flight and ground control experiments performed in 301 hardware and in the 301 capsule are numbered 73-1, 89-1, and 94-1.

We expect that any effect of weightlessness on the genetic effects of radiation will be observed (1) as a significant difference ($P \leq 0.05$) between flight and simultaneous ground control experiments (73-1 vs. 73-2) and (2) as a significant difference ($P \leq 0.05$) between flight experiments and postflight ground control experiments (73-1 vs. 89-1 and 73-1 vs. 94-1). We do not expect significant differences ($P > 0.05$) between any of the ground control experiments (59-2 vs. 73-2, 59-2 vs. 89-2, etc.; 89-1 vs. 89-2 and 94-1 vs. 94-2).

IV. STATUS OF DATA ANALYSIS

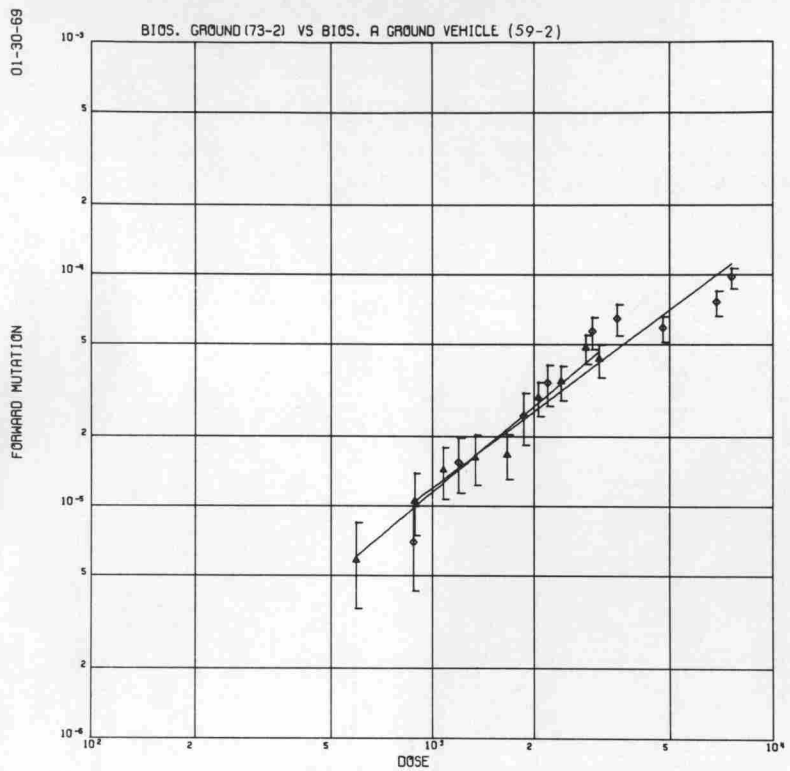
The dose-response curves for the overall induction of ad-3 mutations have been completed for each of the seven experimental designs shown in Table 1. Genetic analysis of the ad-3 mutants is complete only for 59-2, 73-1, and 73-2. The remaining analyses will be complete by April 1969, and comparisons can then be made between data obtained from flight experiments and both postflight ground control experiments.

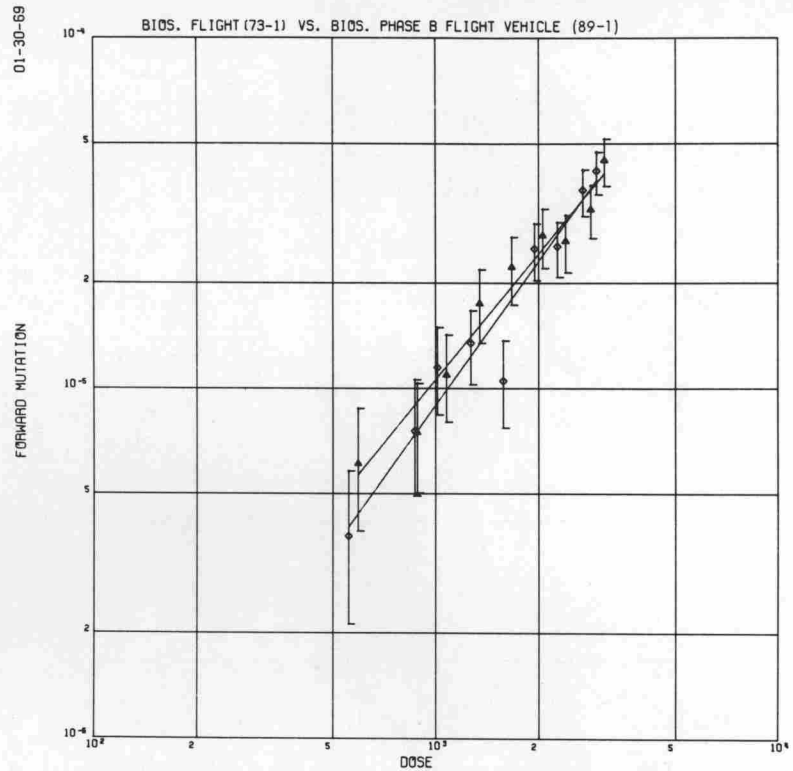
V. COMPARISON OF OVERALL FORWARD-MUTATION CURVES FOR ad-3 MUTATIONS

The primary data output from forward-mutation experiments is the overall induction curve for ad-3 mutations. Comparisons of these curves for each of the experiments listed in Table 1 are given in Fig. 1. In this and subsequent figures comparisons have been made only between

Table 1. Experiment design numbers assigned to each portion of test

Test	Date	Flight Hardware	Ground Control Hardware
Phase A	Dec. 1966	-	59-2
Biosatellite II	Sept. 1967	73-1	73-2
Phase B	Mar. 1968	89-1	89-2
Phase C	May 1968	94-1	94-2

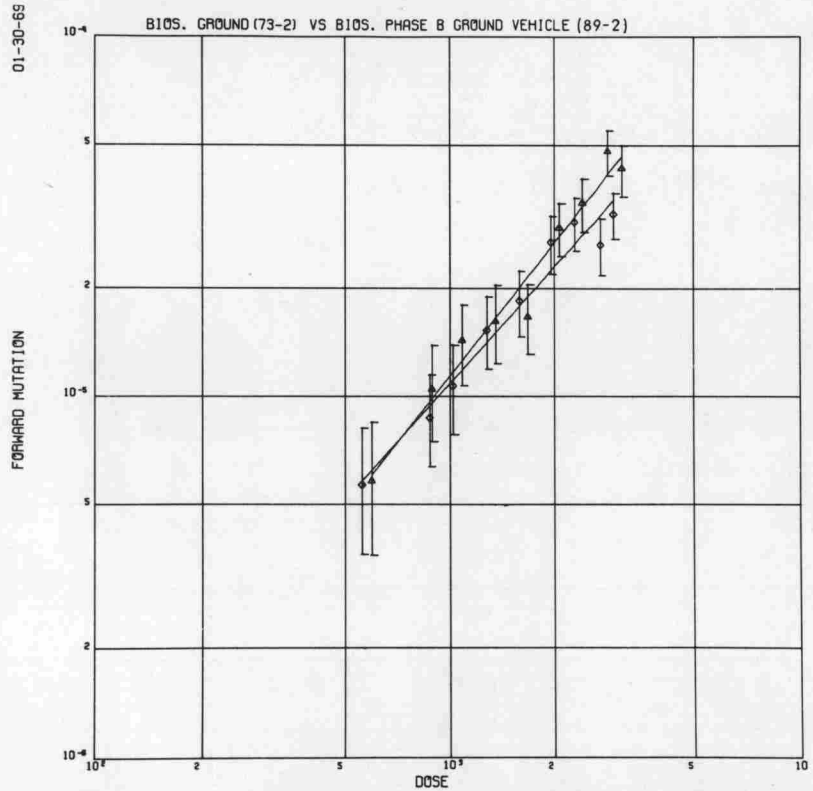


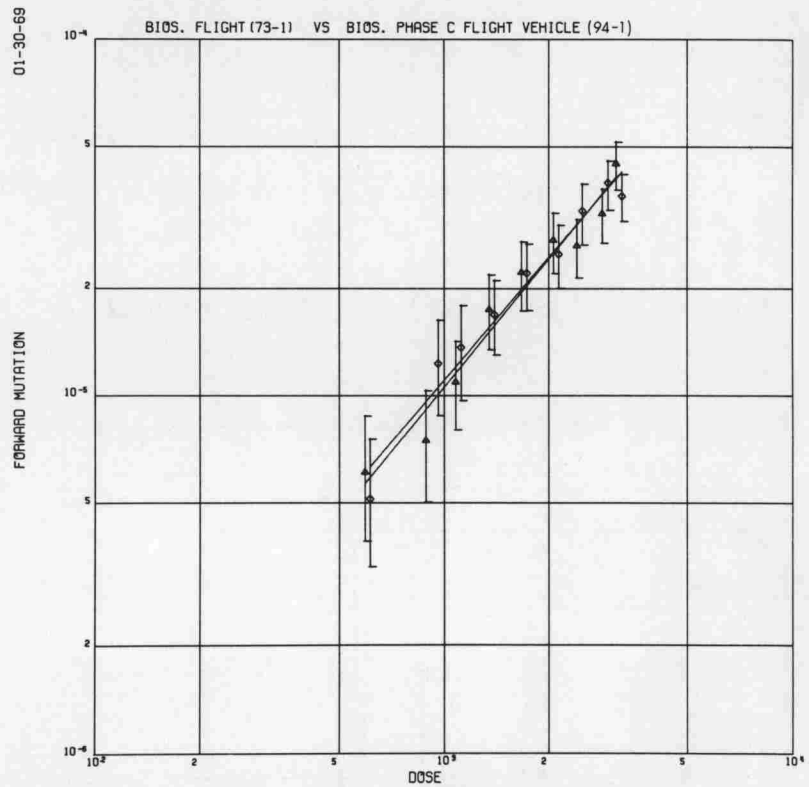


a. 73-2 vs. 59-2 (△ = 73-2, ◇ = 59-2)

b. 73-1 vs. 89-1 (△ = 73-1, ◇ = 89-1)

Fig. 1. Comparisons of Overall Induction Curves for Recessive Lethal Mutations in the ad-3 Region between Flight and Various Ground Control Experiments

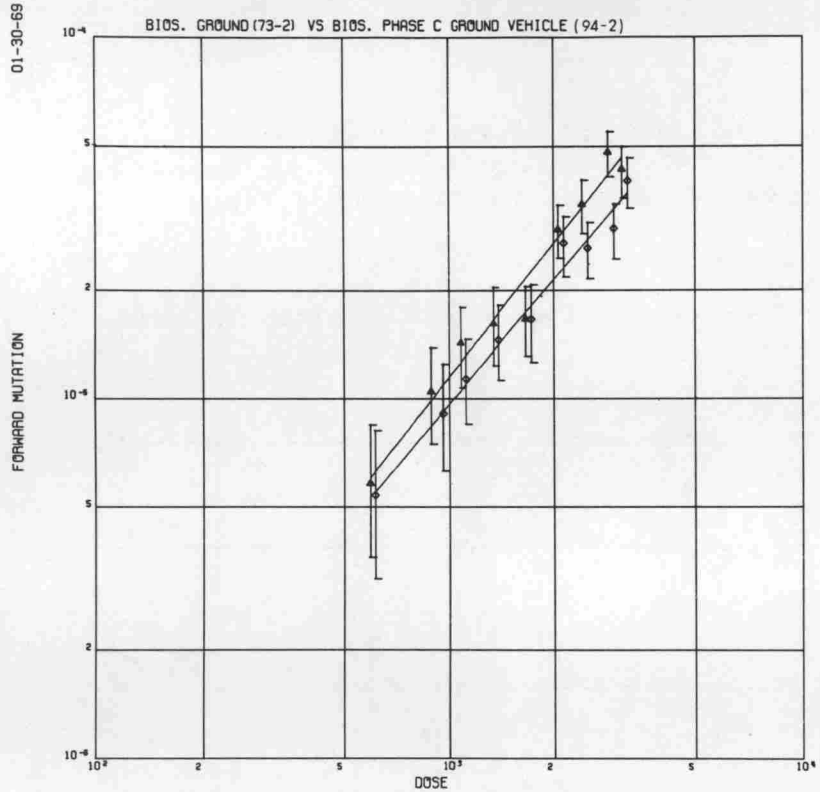


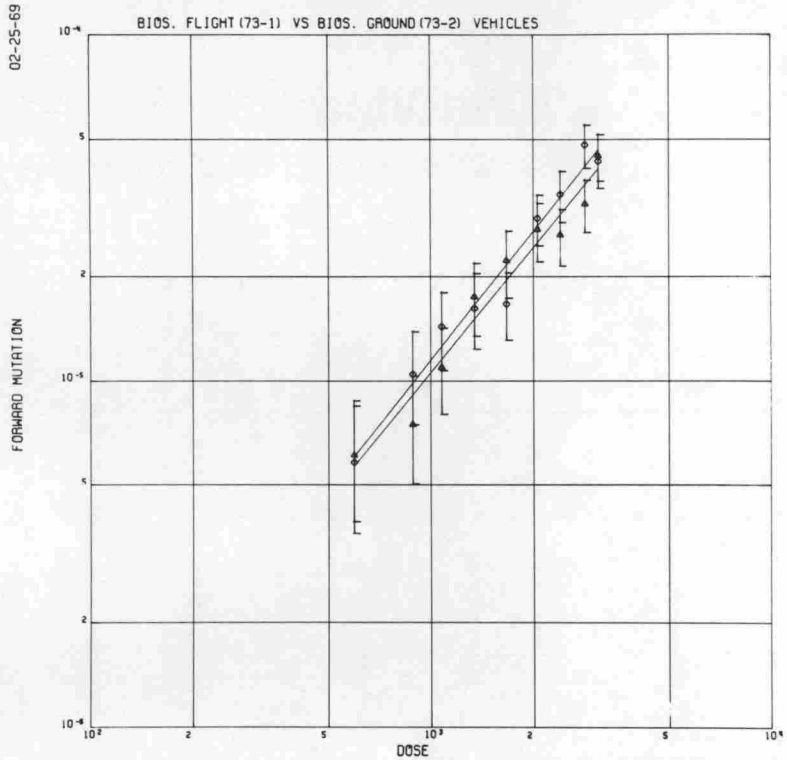


c. 73-2 vs. 89-2 (△ = 73-2, ◇ = 89-2)

d. 73-1 vs. 94-1 (△ = 73-1, ◇ = 94-1)

Fig. 1. Comparisons of Overall Induction Curves for Recessive Lethal Mutations in the ad-3 Region between Flight and Various Ground Control Experiments

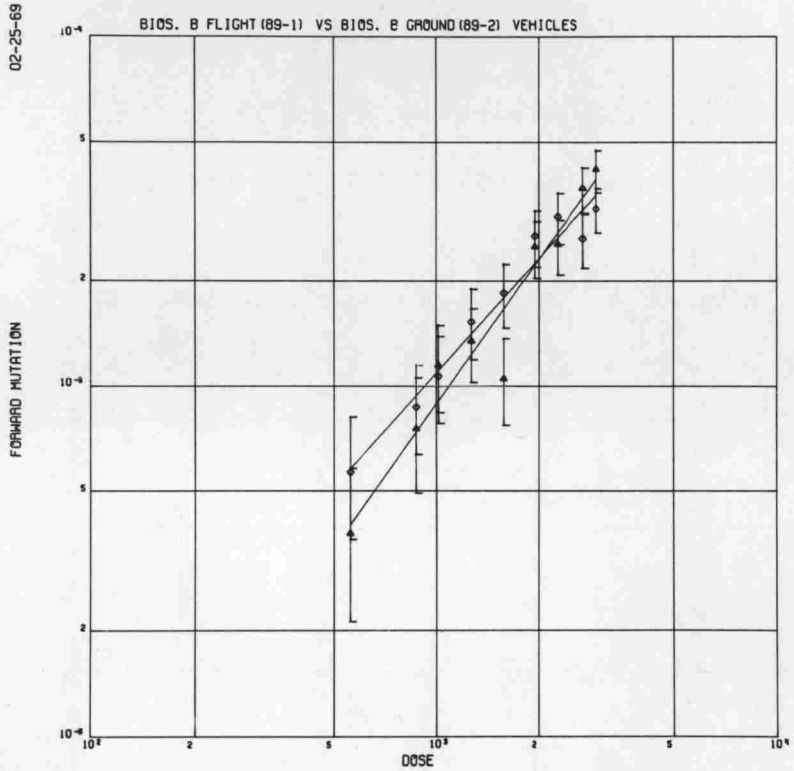


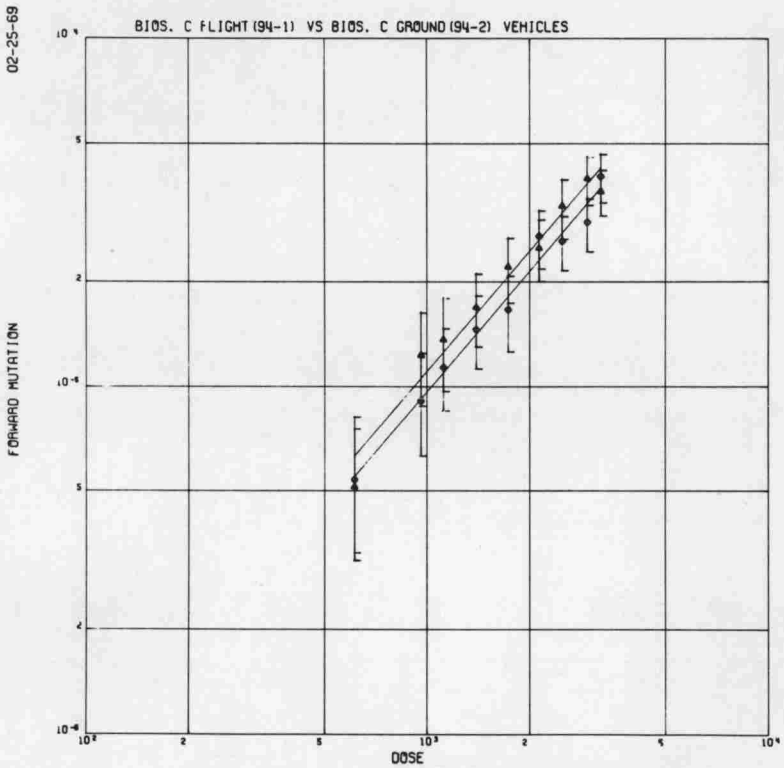


e. 73-2 vs. 94-2 (△ = 73-2, ◇ = 94-2)

f. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)

Fig. 1. Comparisons of Overall Induction Curves for Recessive Lethal Mutations in the ad-3 Region between Flight and Various Ground Control Experiments

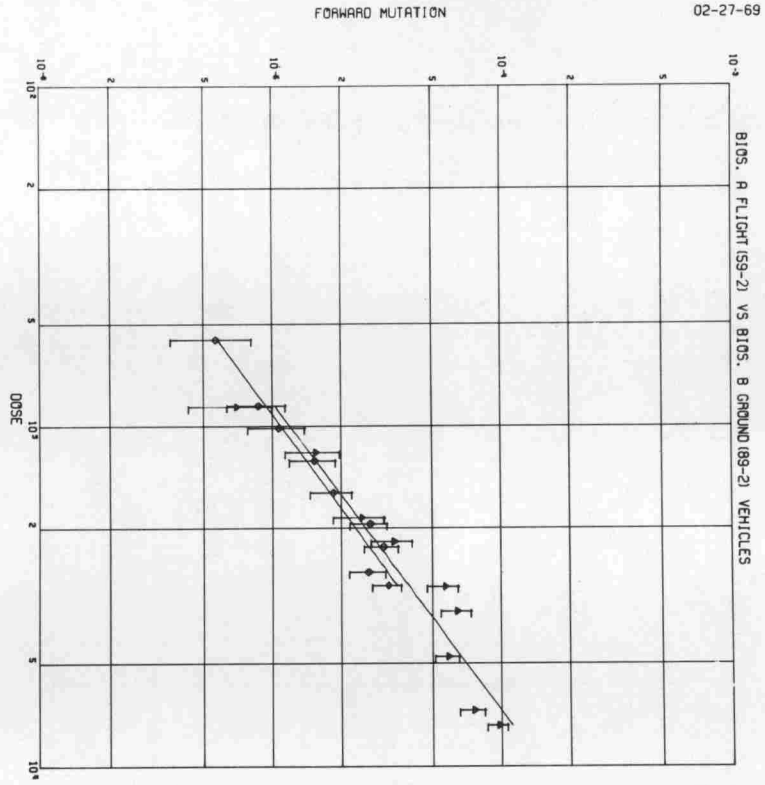




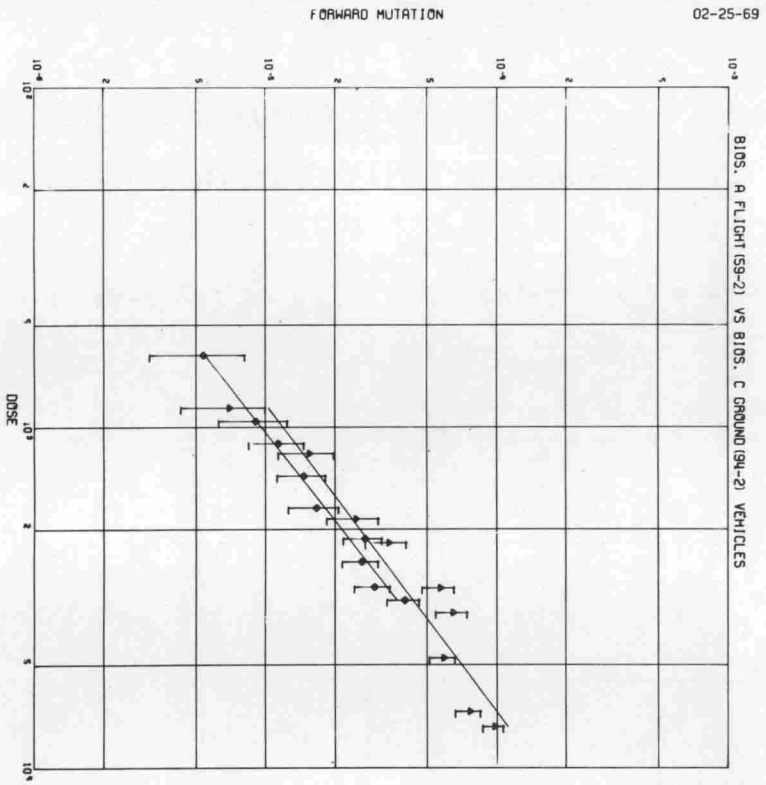
g. 89-1 vs. 89-2 (△ = 89-1, ◇ = 89-2)

h. 94-1 vs. 94-2 (△ = 94-1, ◇ = 94-2)

Fig. 1. Comparisons of Overall Induction Curves for Recessive Lethal Mutations in the ad-3 Region between Flight and Various Ground Control Experiments

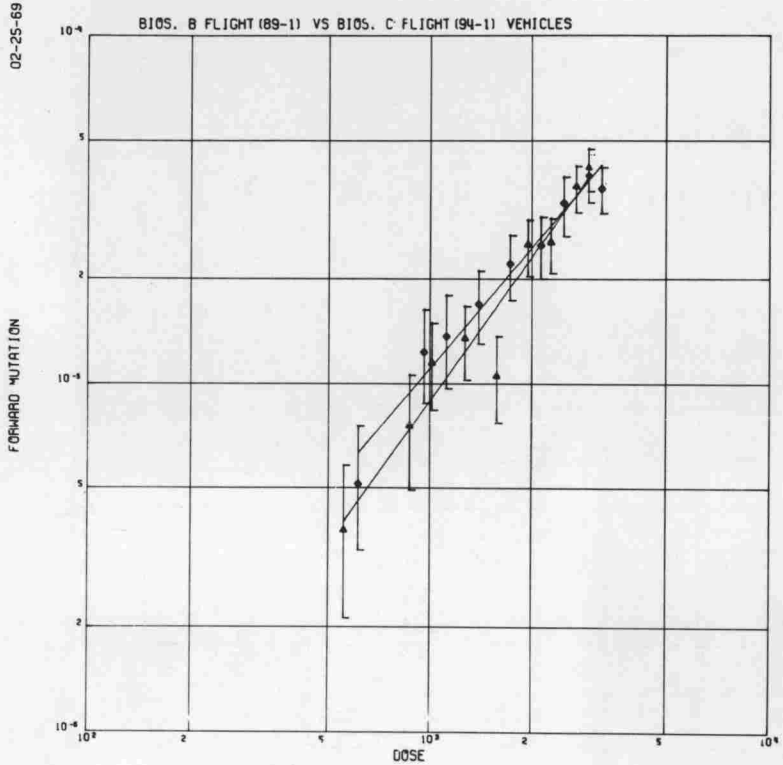


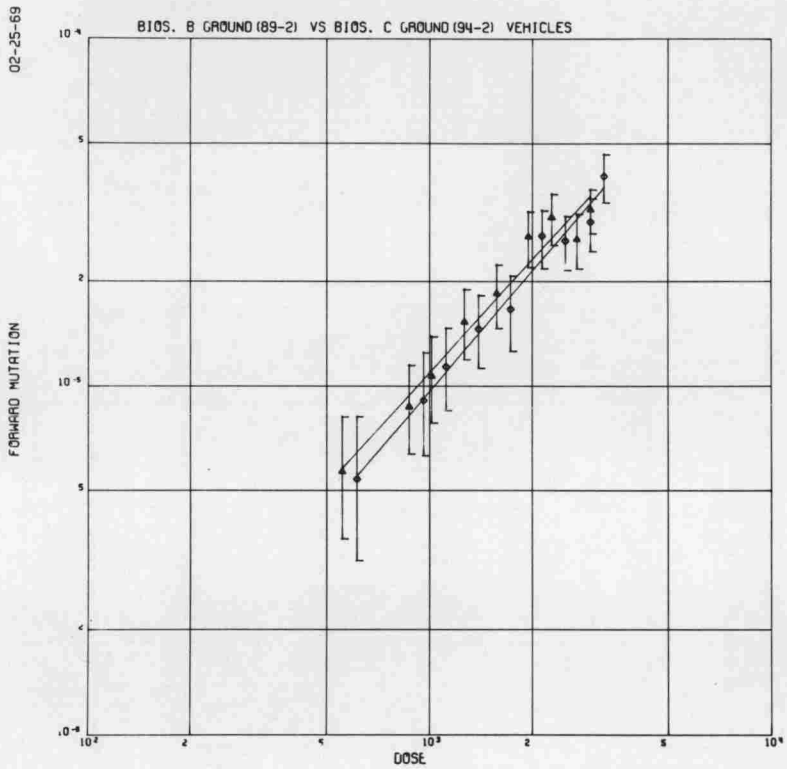
i. 59-2 vs. 89-2 (△ = 59-2, ◇ = 89-2)



j. 59-2 vs. 94-2 (△ = 59-2, ◇ = 94-2)

Fig. 1. Comparisons of Overall Induction Curves for Recessive Lethal Mutations in the ad-3 Region between Flight and Various Ground Control Experiments





k. 89-1 vs. 94-1 (△ = 89-1, ◇ = 94-1)

l. 89-2 vs. 94-2 (△ = 89-2, ◇ = 94-2)

Fig. 1. Comparisons of Overall induction curves for Recessive Lethal Mutations in the ad-3 Region between Flight and Various Ground Control Experiments

dose-response curves obtained with the same set of hardware. The probability of a difference between either the slopes of these curves or the distances between them is given in Table 2. In none of the comparisons is there a significant difference in slope, but there is a significant difference (P = 0.02) in distance between the dose-response curves obtained

Table 2. Probabilities of a difference between slopes and distances in comparisons of the overall forward-mutation curves for ad-3 mutations

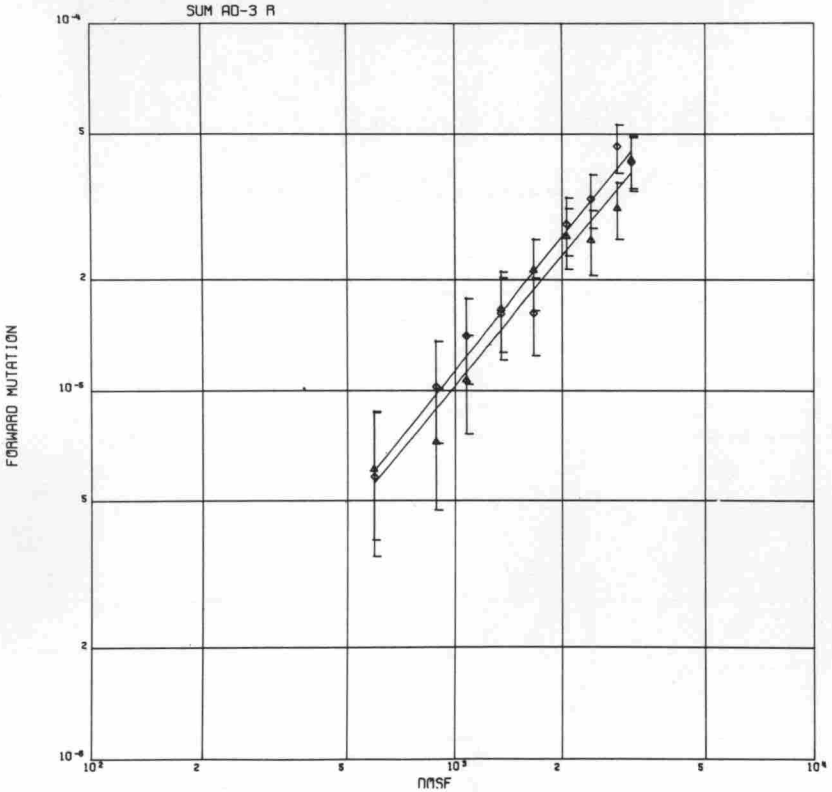
Experimental Design No.	Probability Values	
	Slope	Distance
73-2 vs. 59-2	0.453	0.736
73-1 vs. 89-1	0.301	0.288
73-2 vs. 89-2	0.210	0.102
73-1 vs. 94-1	0.695	> 0.900
73-2 vs. 94-2	0.432	0.002
73-1 vs. 73-2	0.761	0.152
89-1 vs. 89-2	0.089	0.334
94-1 vs. 94-2	> 0.900	0.021
59-2 vs. 89-2	> 0.900	0.375
59-2 vs. 94-2	0.770	0.097
89-1 vs. 94-1	0.184	0.192
89-2 vs. 94-2	0.498	0.099

in the Phase C test (94-1 vs. 94-2). This departure from the expected curve was not due to the data obtained with the flight hardware (94-1 vs. 73-1, 94-1 vs. 89-1) but to the data obtained with the ground control hardware (94-2 vs. 73-2, where P = 0.002; and 94-2 vs. 89-2 and 94-2 vs. 59-2, where P = 0.099 and 0.097, respectively). Forward-mutation frequencies obtained with the control hardware were significantly lower in experiment 94-2 than in any other test where this hardware was used: 59-2, 73-2, and 89-2. It is important to note that the differences observed between these experiments are small.

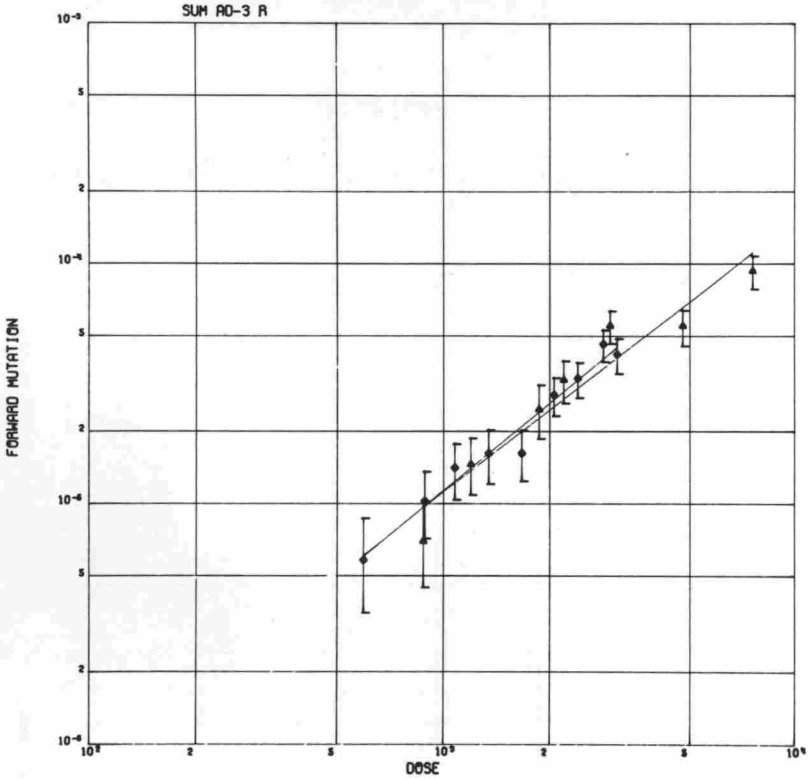
VI. COMPARISON OF DOSE-RESPONSE CURVES OF INDIVIDUAL COMPONENTS OF OVERALL FORWARD-MUTATION CURVES

Genetic analysis of the ad-3 mutations has been completed for only three of the seven experimental designs listed in Table 1: 59-2, 73-1, and 73-2. These three analyses make it possible to compare the dose-response curves of the individual components of the overall curves. It is entirely possible to have differences between the components when there is no difference between the overall curves.

In theory, data from the ground control experiment (59-2 vs. 73-2) should agree, whether or not there is any difference between the flight experiments and simultaneous ground controls (73-1 vs. 73-2). Comparisons of the overall dose-response curves for point mutations, $ad\text{-}3^{R}$, and the overall curves for chromosome deletions, $ad\text{-}3^{IR}$, are given in Fig. 2. Comparisons of the dose-response curves for point mutations at the ad-3A

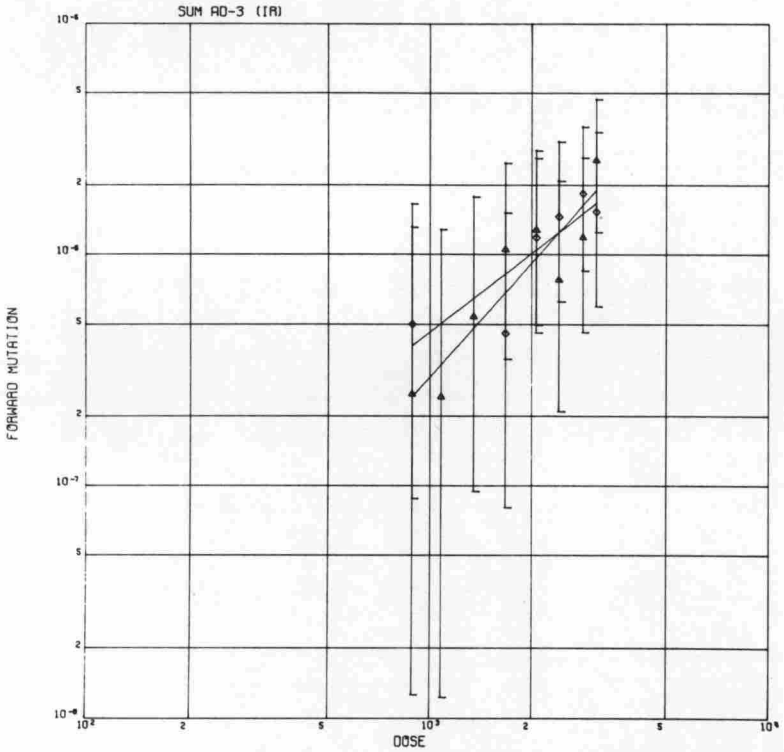


a. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)

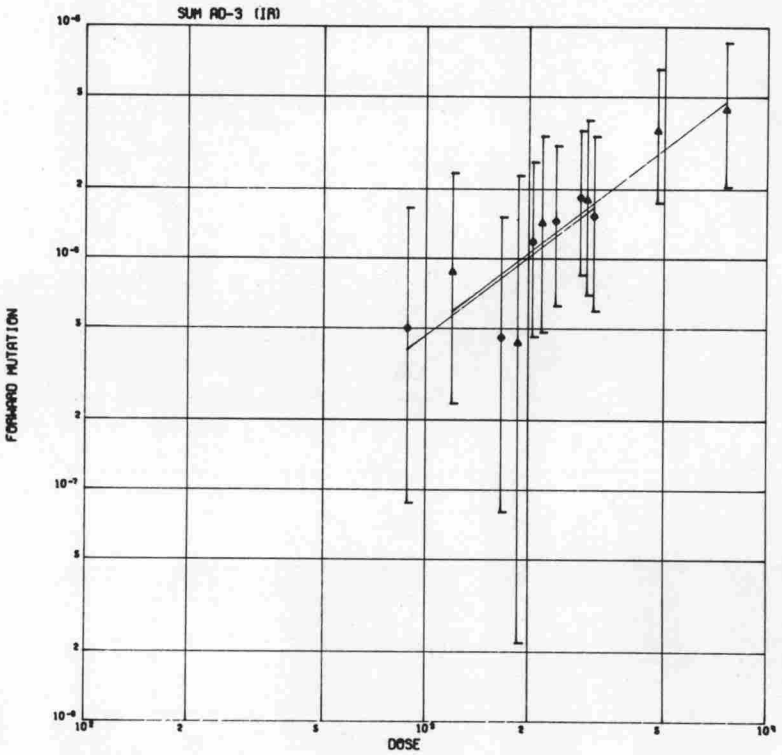


b. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 2. Comparisons of the Induction Curves for $ad\text{-}3^{R}$ Mutations and $ad\text{-}3^{IR}$ Mutations between Flight and Various Ground Control Experiments



c. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)

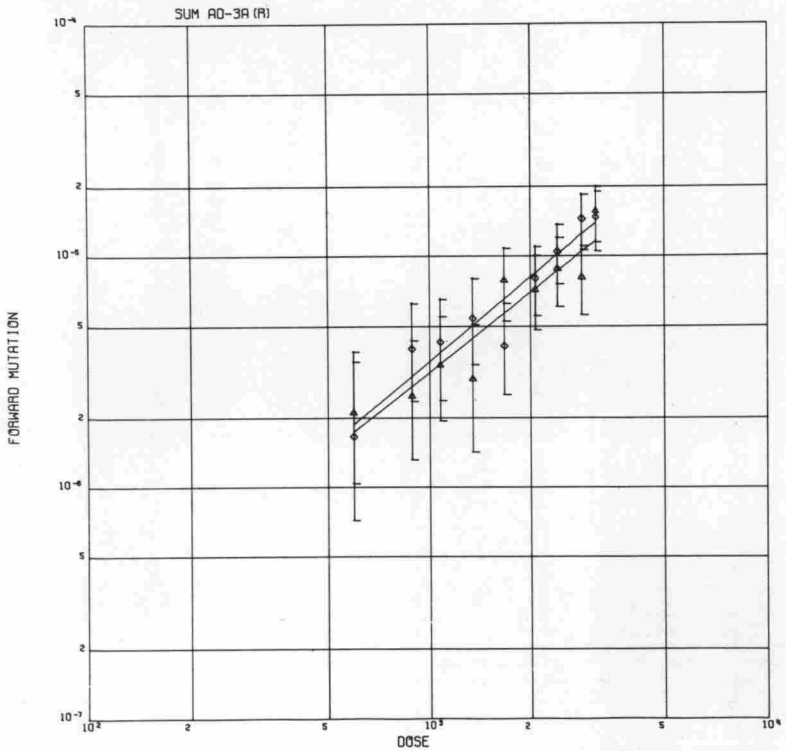


d. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

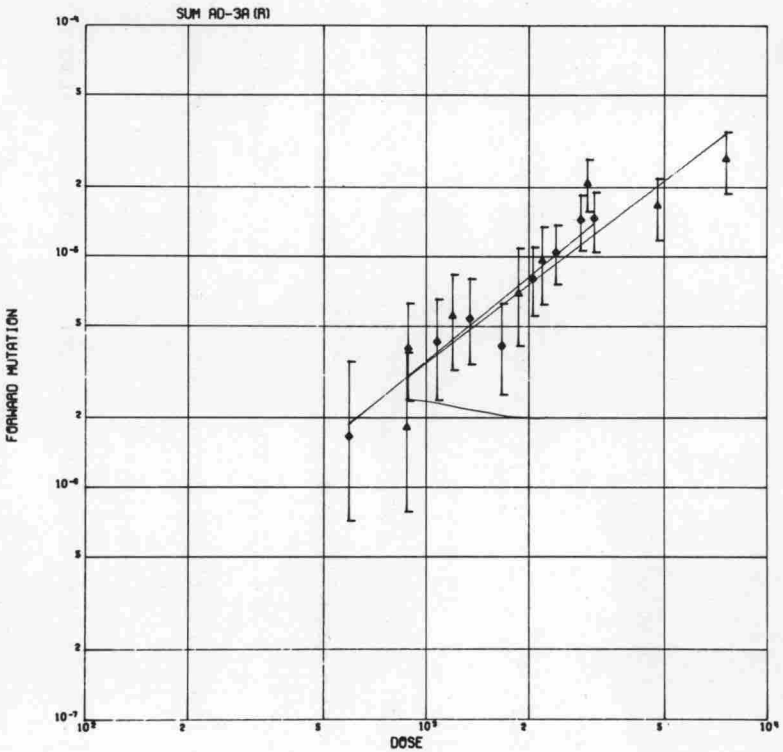
Fig. 2. Comparisons of the Induction Curves for ad-3^{R} Mutations and ad-3^{IR} Mutations between Flight and Various Ground Control Experiments

locus, ad-3A^{R}, and point mutations at the ad-3B locus, ad-3B^{R}, are given in Fig. 3. Comparisons of the dose-response curves for chromosome deletions covering the ad-3A locus, ad-3A^{IR}, the ad-3B locus, ad-3B^{IR} or both loci simultaneously, (ad-3A ad-3B)^{IR}, are given in Fig. 4. Comparisons of the dose-response curves for each type of complementation pattern among point mutations at the ad-3B locus--nonpolarized, ad-3B^{R-NP}; polarized, ad-3B^{R-P}; and noncomplementing, ad-3B^{R-NC}--are given in Fig. 5. The probabilities of a difference between the slopes of these curves or the distance between them are given in Table 3. These comparisons show that there is a significant difference ($P \leq 0.05$) between the flight and ground control samples only with regard to noncomplementing ad-3B mutants, ad-3B^{R-NC}, where the distance between the dose-response curves is significant ($P = 0.01$). These comparisons also show that there is no difference between the spectra of ad-3 mutations in the control portions of these two experiments (59-2 vs. 73-2) except for ad-3B^{IR} mutations, where the distance between the dose-response curves is significant. Until comparisons can be made with the two postflight control experiments we will not know whether the frequency of ad-3B^{IR} mutations is unusual in experiment 59-2 or 73-2.

The difference between the dose-response curves for ad-3B^{R-NC} mutants in the comparison 73-1 vs. 73-2 results in a difference in percentage allelic complementation as well as in types of complementation patterns (see below).

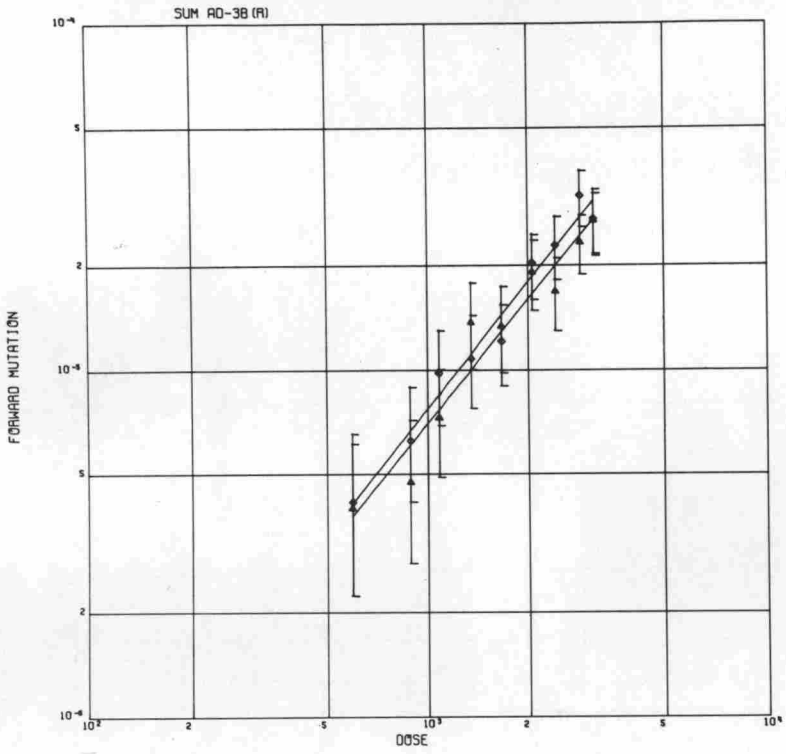


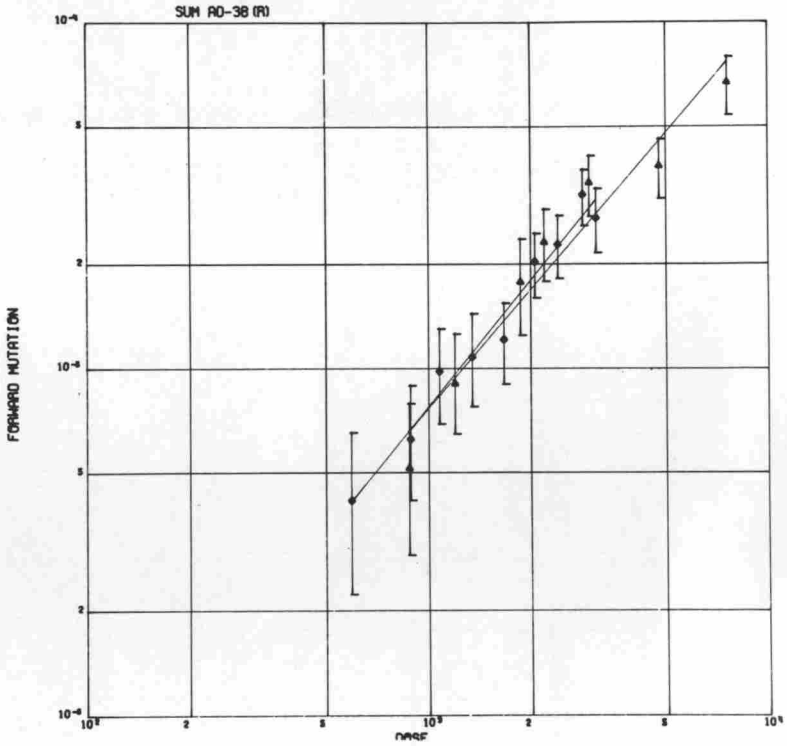
a. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)



b. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 3. Comparisons of Induction Curves for ad-3A^R Mutations and ad-3B^R Mutations between Flight and Various Ground Control Experiments

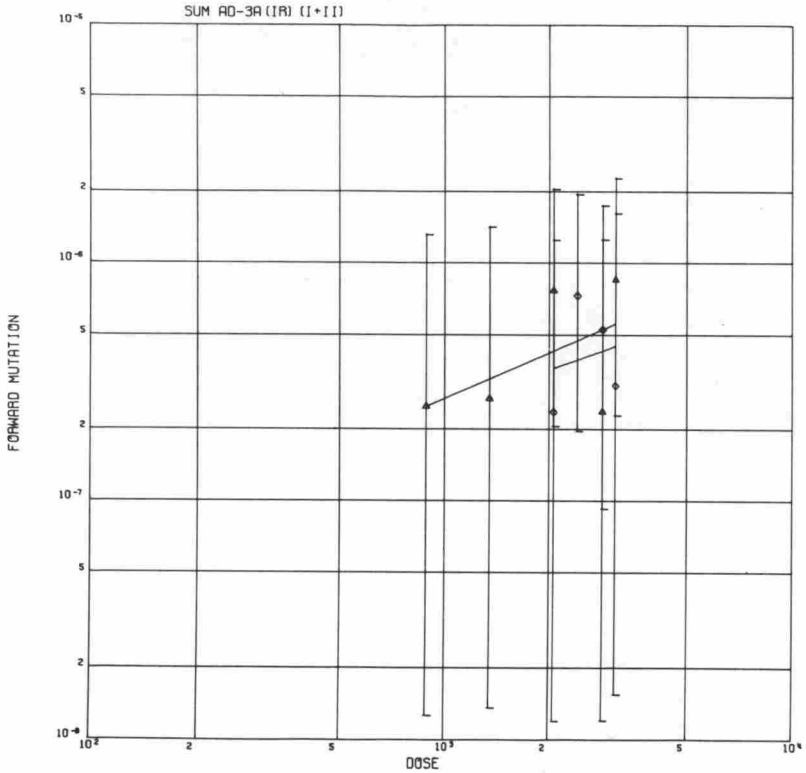




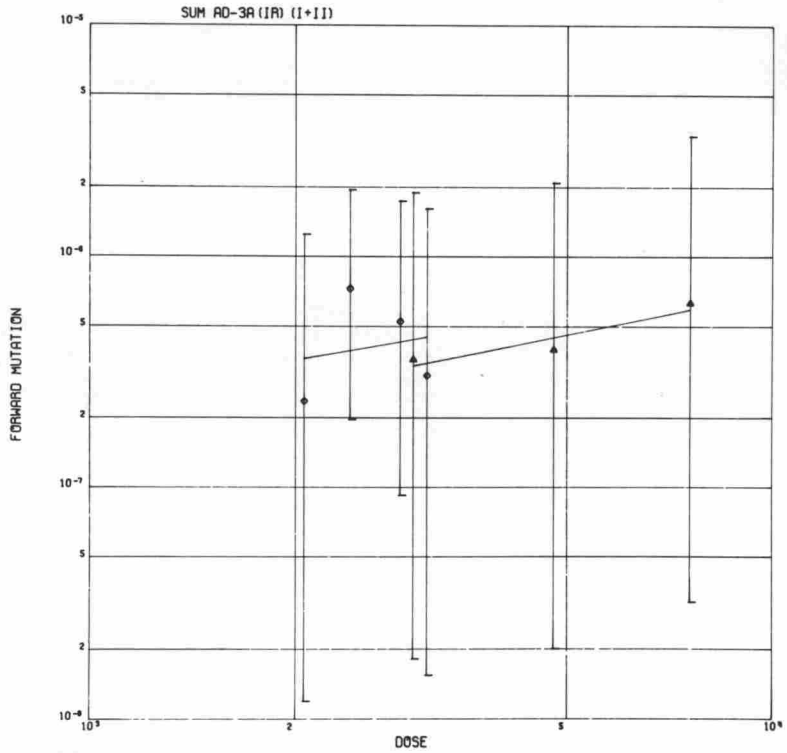
c. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)

d. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 3. Comparisons of Induction Curves for ad-3A^R Mutations and ad-3B^R Mutations between Flight and Various Ground Control Experiments

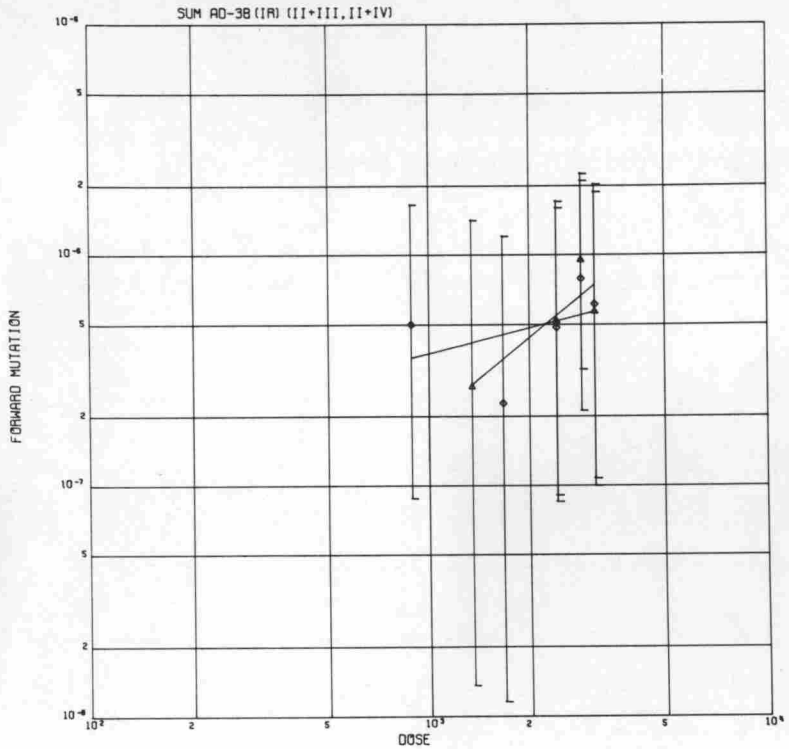


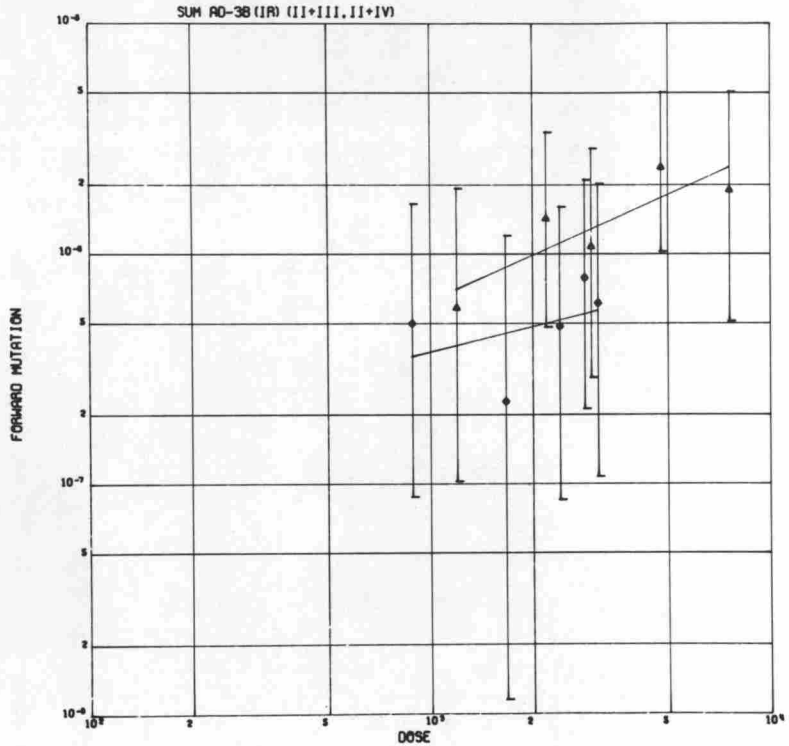
a. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)



b. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 4. Comparisons of Induction Curves for ad-3A^{IR}, ad-3B^{IR}, and (ad-3A ad-3B)^{IR} Mutations between Flight and Various Ground Control Experiments

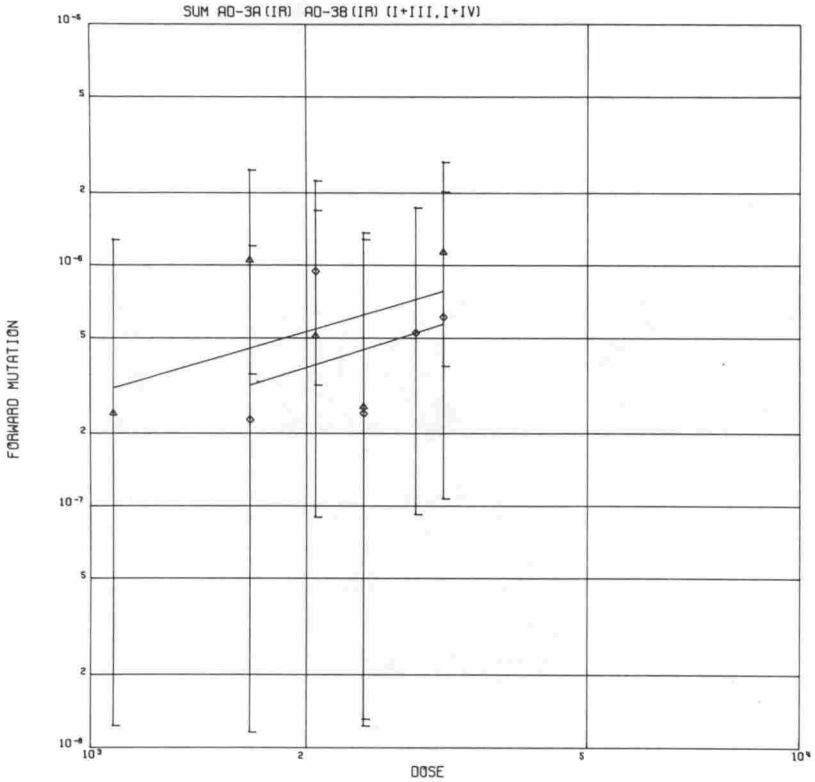




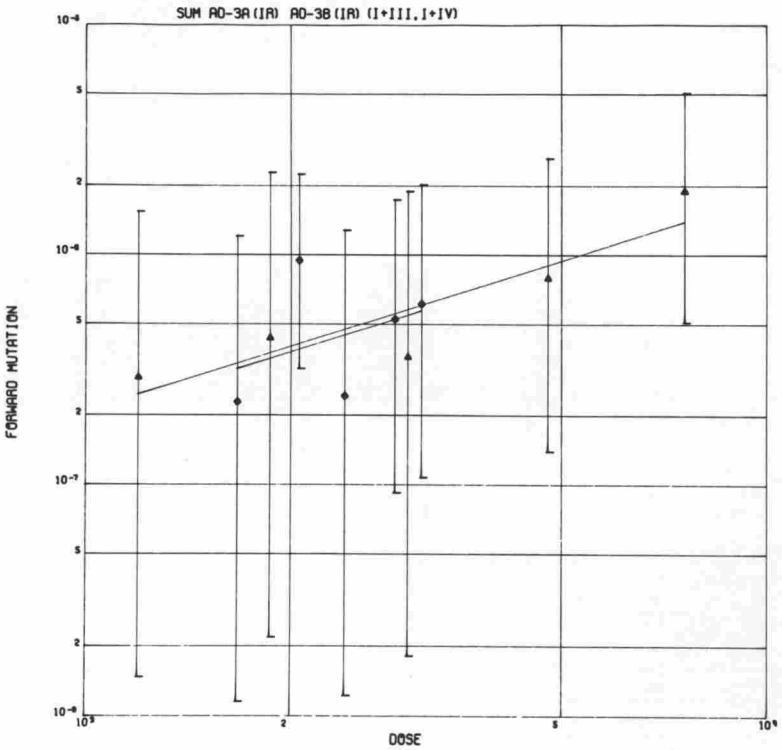
c. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)

d. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 4. Comparisons of Induction Curves for ad-3A^{IR}, ad-3B^{IR}, and (ad-3A ad-3B)^{IR} Mutations between Flight and Various Ground Control Experiments

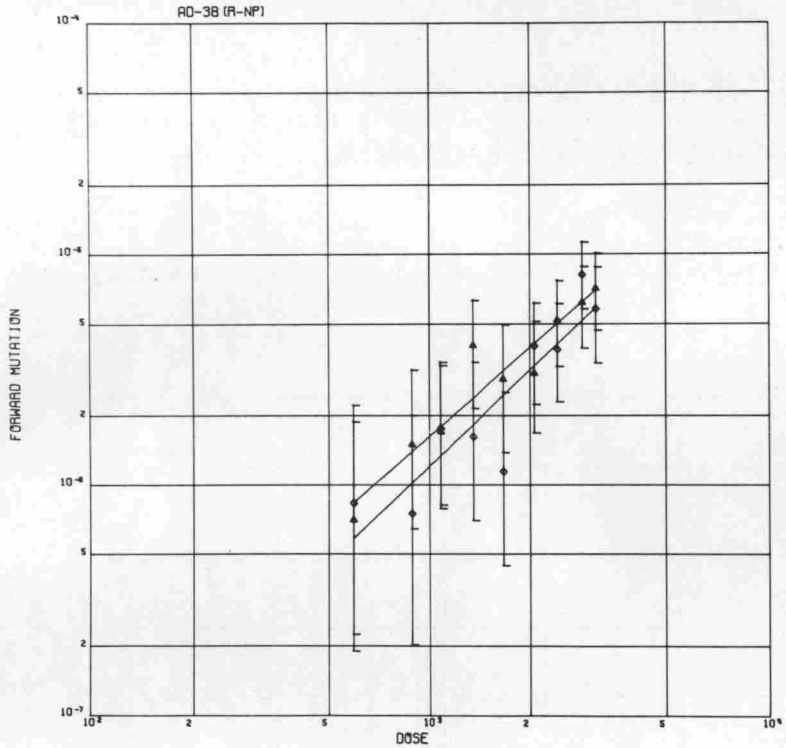


e. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)

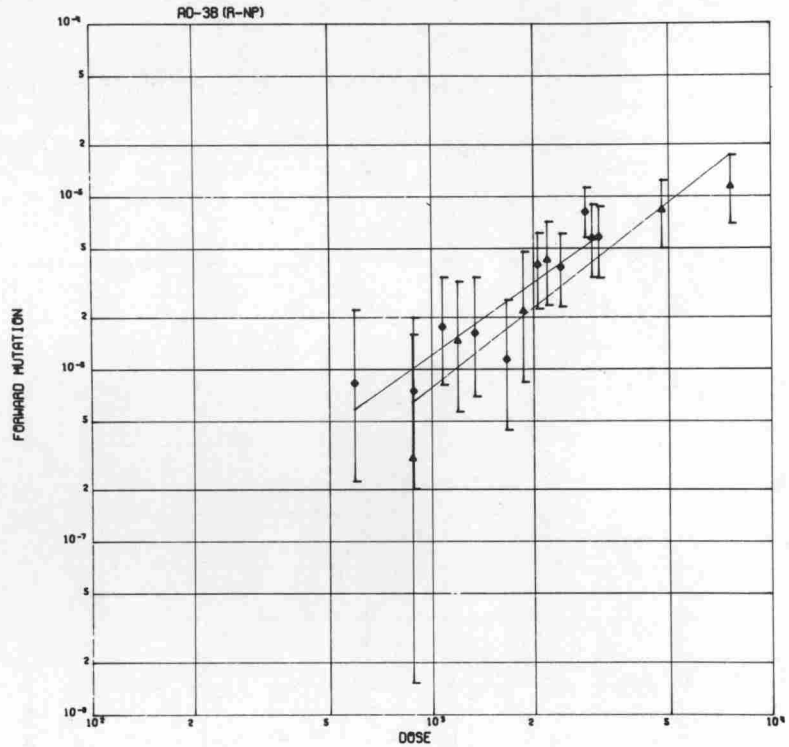


f. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 4. Comparisons of Induction Curves for ad-3A^{IR}, ad-3B^{IR}, and (ad-3A ad-3B)^{IR} Mutations between Flight and Various Ground Control Experiments

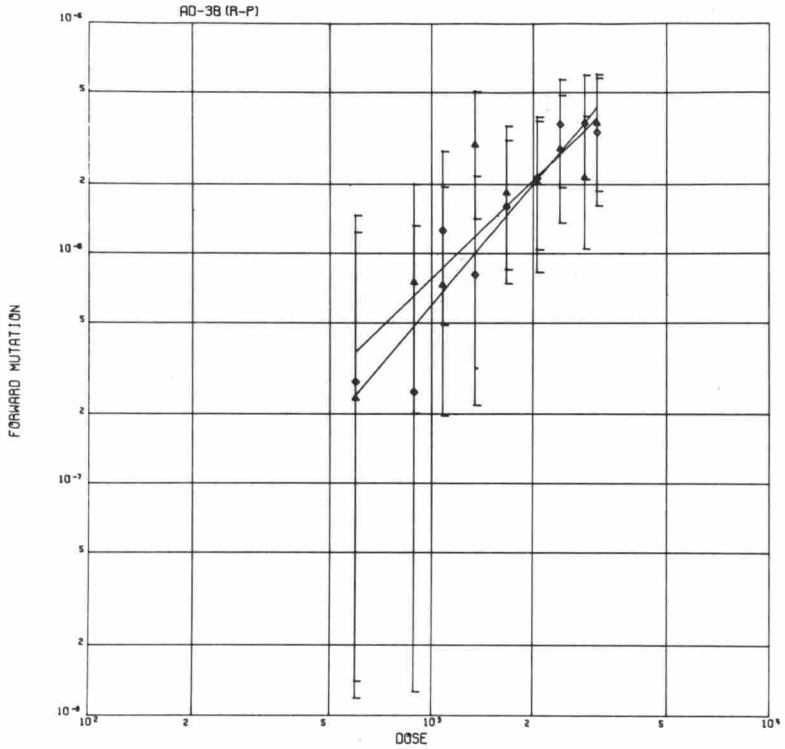


a. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)

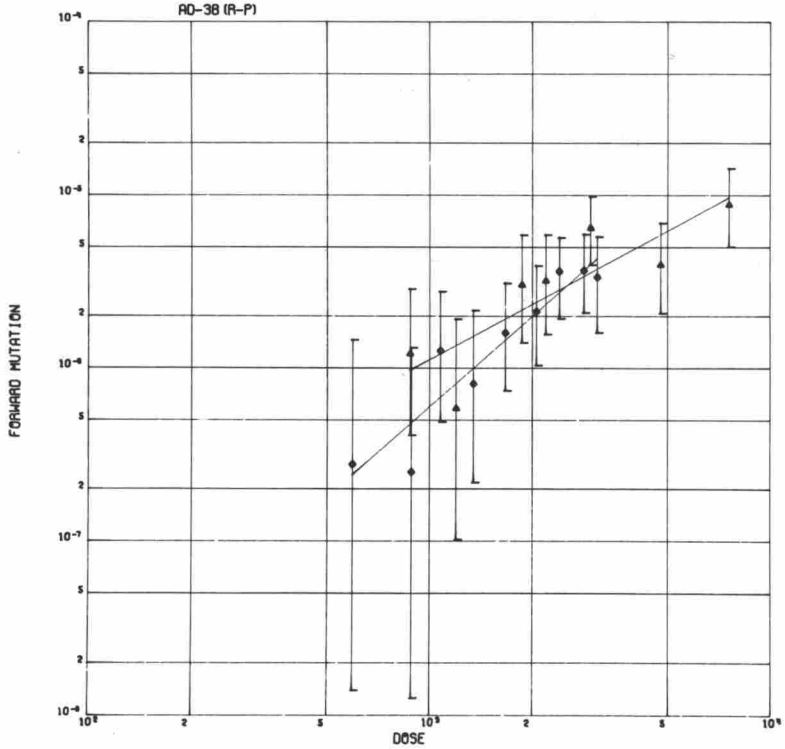


b. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 5. Comparisons of the Induction Curves for ad-3B^{R-NP}, ad-3B^{R-P}, and ad-3B^{R-NC} Mutations between Flight and Various Ground Control Experiments

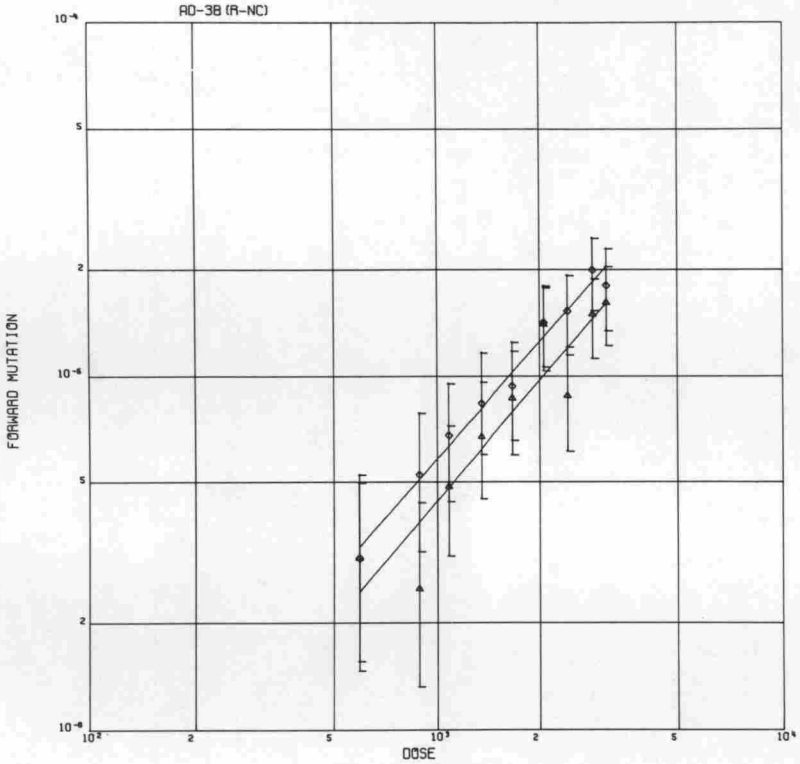


c. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)

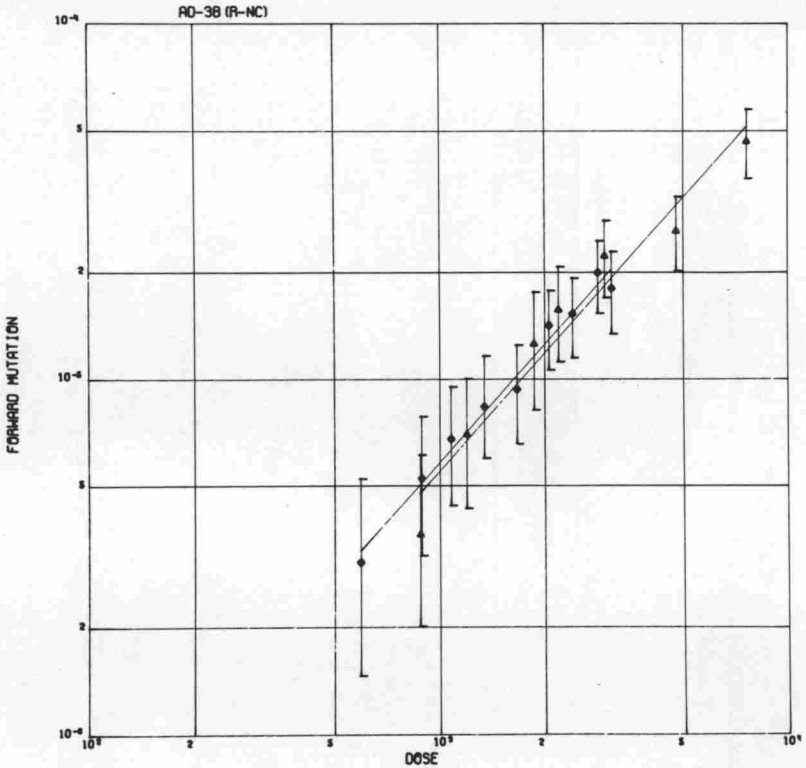


d. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 5. Comparisons of the Induction Curves for ad-3B^{R-NP}, ad-3B^{R-P}, and ad-3B^{R-NC} Mutations between Flight and Various Ground Control Experiments



e. 73-1 vs. 73-2 (△ = 73-1, ◇ = 73-2)



f. 59-2 vs. 73-2 (△ = 59-2, ◇ = 73-2)

Fig. 5. Comparisons of the Induction Curves for ad-3B^{R-NP}, ad-3B^{R-P}, and ad-3B^{R-NC} Mutations between Flight and Various Ground Control Experiments

Table 3. Probabilities of a difference between slopes and distances in comparisons of the individual components of the overall induction curve for ad-3 mutants

Experimental Design No.	Probability Values	
	Slope	Distance
I. $ad\text{-}3^{R}$		
73-1 vs. 73-2	0.773	0.095
59-2 vs. 73-2	0.646	0.603
$ad\text{-}3^{IR}$		
73-1 vs. 73-2	0.292	0.552
59-2 vs. 73-2	> 0.900	0.858
II. $ad\text{-}3A^{R}$		
73-1 vs. 73-2	0.776	0.246
59-2 vs. 73-2	0.752	0.684
$ad\text{-}3B^{R}$		
73-1 vs. 73-2	0.856	0.161
59-2 vs. 73-2	0.612	0.588
III. $ad\text{-}3A^{IR}$		
73-1 vs. 73-2	> 0.900	0.665
59-2 vs. 73-2	> 0.900	0.624
$ad\text{-}3B^{IR}$		
73-1 vs. 73-2	0.342	0.900
59-2 vs. 73-2	0.572	0.028
$(ad\text{-}3A\ ad\text{-}3B)^{IR}$		
73-1 vs. 73-2	> 0.900	0.465
59-2 vs. 73-2	> 0.900	0.869
IV. $ad\text{-}3B^{R\text{-}NP}$		
73-1 vs. 73-2	0.718	0.127
59-2 vs. 73-2	0.704	0.166
$ad\text{-}3B^{R\text{-}P}$		
73-1 vs. 73-2	0.389	0.554
59-2 vs. 73-2	0.099	0.457
$ad\text{-}3B^{R\text{-}NC}$		
73-1 vs. 73-2	0.845	0.010
59-2 vs. 73-2	> 0.900	0.538

VII. COMPARISON OF FREQUENCIES OF DIFFERENT COMPLEMENTATION PATTERNS AMONG ad-3B MUTANTS

Ad-3B mutants show allelic complementation and are either complementing with nonpolarized (NP) or polarized (P) complementation patterns, or are noncomplementing (NC). Since there is a correlation between complementation pattern and genetic alteration at the molecular level, a difference in the percentages of mutants with each type of pattern is interpreted as presumptive evidence for a difference at the molecular level. The percentages of mutants with NP, P, and NC patterns in the flight and simultaneous ground control experiments (73-1 vs. 73-2) are shown in Table 4. A chi-square test for a difference between these distributions shows a significant difference (P = 0.035), but no difference was found between the simultaneous ground control and the Phase A ground control (73-2 vs. 59-2) in a similar chi-square test for a difference in the two distributions of complementation patterns (P = 0.396).

This analysis shows clearly that there are significantly different distributions of complementation patterns between the flight and ground control experiments.

Table 4. Chi-square tests for homogeneity on the distribution of complementation patterns in flight and ground control samples of $ad\text{-}3B^R$ mutants

Expt. No.	No. of Mutants			Expt. No.	No. of Mutants			Probability Level
	NP	P	NC		NP	P	NC	
73-1	125	66	310	73-2	107	66	389	0.035
59-2	78	64	311	73-2	107	66	389	0.396

VIII. DISCUSSION

Insofar as the analysis of the experimental material from the four experiments listed in Table 1 has proceeded, we can see that there is no difference between the overall induction curves for ad-3 mutations in the flight and simultaneous ground control experiments (73-1 vs. 73-2). Nor is there a difference between the flight and ground control experiments performed in flight hardware (73-1 vs. 89-1, and 73-1 vs. 94-1). But the difference observed between the two sets of data in the Phase C experiment (94-1 vs. 94-2) introduces an unexpected complication, since the difference is apparently due to the fact that significantly lower forward-mutation frequencies were found in the ground-control hardware portion of this experiment (94-2). Although at present we cannot explain why the sensitivity of the heterokaryotic conidia to induction of ad-3 mutations was lower than expected in this portion of experiment 94, we should be able to evaluate this situation more completely when the characterization of the ad-3 mutants has been completed and comparisons of the dose-response curves for the individual components of the overall ad-3 mutation curves have been made.

Nevertheless, the comparison of the flight data with ground control data, insofar as these comparisons can be made, indicate that there is little, if any, effect of weightlessness on the spectrum of radiation-induced ad-3 mutations.

Essentially the same experiment was performed with the Neurospora assay system on the Gemini XI mission. In that portion of the experiment where Neurospora samples were flown collected on the surface of Millipore

filters, no difference was found between the flight and ground control samples (de Serres et al., 1969; de Serres and Smith, 1969). Thus we have a completely independent confirmation of our Biosatellite data.

It is of considerable interest, however, that the Neurospora experiment was also flown in suspension on the Gemini XI mission and that a significant difference was found between flight and ground control samples. The combined data on the Neurospora experiment show clearly that there is an effect of weightlessness on radiation-induced recessive lethal mutations, but that the level of the effect is related to the metabolic activity of the conidia. Little or no effect was found with dormant spores collected on the surface of Millipore filters, but an effect was found with metabolically active spores in suspension.

IX. SUMMARY

Genetic analyses of the flight and ground control portions of the Neurospora experiment on the Biosatellite II mission show that there is little or no effect of weightlessness on radiation-induced recessive lethal mutations on metabolically inactive spores.

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