学位申請論文

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主 論 文

Comparative Analysis of Lethal Lesions Produced by P^{32} Decay and γ-Rays in Phage T1 and in its Host Strains of *Escherichia coli* with Normal and Reduced Repair Abilities[1]

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Running head: P^{32} and γ-Ray Lethal Lesions

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ABSTRACT

IKENAGA, M. Comparative Analysis of Lethal Lesions Produced by P^{32} Decay and γ-Rays in Phage T1 and in its Host Strains of Escherichia coli with Normal and Reduced Repair Abilities. Radiation Res. __, pp. __ - __ (196).

Phage T1 in vitro irradiated by γ-rays in broth was inactivated when plated on radiation sensitive strains B_{s-1} and R11 of Escherichia coli at 1.2 times higher rate than when plated on resistant strains H/r30 and H/r30-R, whereas when inactivated in vitro by incorporated P^{32} it gave exactly the same number of plaques on B_{s-1} as on H/r30.

Sensitivity to killing of host strains H/r30, R11 and B_{s-1} were in the ratios 1 : 1.2 : 4.0 for aerobic γ-ray irradiation, 1 : 1.2 : 6.3 for anoxic γ-ray irradiation, 1 : 1 : 2 for aerobic P^{32} treatment and 1 : 1 : 2.2 for anoxic P^{32} treatment.

Ultracentrifugation analysis of P^{32}-incorporated DNA of T1 supported the assumption that one lethal hit approximately corresponds to one double-strand break. Based on this evidence, the inactivation data for phage and E. coli were analyzed. It was concluded that P^{32} killing of T1 is primarily caused by transmutation of P^{32}, whereas P^{32} killing of host bacterium is only partly caused by transmutation. It was estimated that transmutations are responsible for about 40 and 70 % of lethal lesions induced by P^{32} decay in strains B_{s-1} and H/r30, respectively, the rest being caused by β-rays.

P^{32} effects on T1 phage and E. coli; P^{32} induced DNA strand break; repair of P^{32} and γ-ray lesions.

INTRODUCTION

There is a considerable amount of evidence that the primary cause of killing by ultraviolet light (UV) in microorganisms is due to the formation of pyrimidine dimers. It is also well established that the normal strains of Escherichia coli possess enzymatic mechanisms for repair of UV lesions. One of the best understood mechanisms is the "excision repair" system, which excises the dimers from DNA molecules and resynthesizes the excised regions (1, 2). But, not much is yet known about the nature of primary X-ray damage and its repair mechanism in spite of many interesting and important findings by many workers (3-8).

Stent and Fuerst (9) have suggested that inactivation of phage by disintegration of P^{32} incorporated into DNA is due to double-strand breaks. The double-strand breaks coincide the only about one-tenth of single-strand breaks that are produced at each event of P^{32} decay (10). Recently, Freifelder (11) has shown with a centrifugation method that when coli phage T7 is irradiated by X-rays in an inorganic buffer, inactivation of the phage is almost entirely due to double-strand breaks produced in DNA molecules.

This paper reports:

(1) Centrifugation data of P^{32}-incorporated DNA of T1 phage. It is concluded that the number of lethal hits induced by P^{32} in T1 equals the number of double-strand breaks per DNA molecule.

(2) On the reparable fractions of DNA lesions induced by P^{32} and γ-rays in T1 phage. This was studied by analysis of phage survival on a normal host strain as compared to a γ-ray sensitive strain.

(3) P^{32} and γ-ray sensitivities of E. coli. The E. coli strains

used as host for T1 phage were subjected to comparative studies of their sensitivities to P^{32} and γ-rays in air and nitrogen atmosphere.

The experimental data of T1 phage and their analysis permit certain conclusions concerning difference in inactivation characters by P^{32} and γ-ray damages on T1 DNA. These conclusions were then applied to P^{32} killing of E. coli to estimate separately the fraction of lethal lesions due to the transmutation of P^{32} from that due to concomitant β emission. The β-rays accompany the decay of P^{32} in DNA and in other cellular constituents.

MATERIALS AND METHODS

Bacterial Strains

The radio-resistant strains of E. coli used are B/r30 and its photoreactivable variant B/r30-R (of B/r type with the normal ability of host cell reactivation, HCR). They were isolated by Witkin (12) from Harm's non-photoreactivable mutant (13) of E. coli B. A radio-sensitive mutant used was E. coli B_{s-1} (3) which has a reduced ability to propagate the UV irradiated T1 phage (hcr^-; see reference (2)). Another sensitive mutant, R11, isolated from B/r30-R in Dr. Kondo's laboratory was also employed. The R11 shows the same hcr^- character as B_{s-1} but it is not so sensitive as B_{s-1} to the loss of colony forming ability by UV and X-rays (14).

Media

Unless otherwise stated, 1 % of Difco nutrient broth with 0.5 % NaCl added was used for ordinary culture of non-radioactive bacteria. Solid medium consisted of 10 gm of polypeptone, 3 gm of yeast extract, 2.5 (mg) of KCl, 15 gm of agar and 1 liter of distilled water, adjusted

to pH 7.2 by NaOH. For dilution of bacteria, buffered saline was used (1/15 M phosphate buffer plus 0.1 M NaCl). The buffered saline was tenfold diluted by distilled water for T1 phage experiments.

Survival Assay for Bacteria and Phage

Aliquots of the bacterial suspension were spread on agar plates and colonies were counted after 16 to 18 hours incubation at 37°C.

The agar layer technique described by Adams (15) was used for phage assay. Host bacteria were grown in aerated nutrient broth to a concentration of about 2×10^8 cells/ml, then centrifuged and resuspended in T1 buffer (see Media) at a titer of about 2×10^9 cells/ml. Finally, they were starved for 30 min at 37°C with aeration to get reproducible data. Adsorption of T1 phage was performed in T1 buffer for 15 min at 37°C.

Preparation of Radioactive Bacteria and Phage

Bacteria in the exponential phase of growth in non-radioactive glycerol lactate medium (H medium)(9) were transferred to radioactive H medium containing the desired amount of carrier-free P^{32} (The Radiochemical Centre, England) and growth was continued for about 4 hours. The P^{32} labeled bacteria at a titer of about 2×10^8 cells/ml were washed twice with cold buffer by centrifugation and then resuspended in ice-cold glycerol casaminoacid medium (GCA medium)(16). One-ml aliquots were sealed into 2 ml glass ampoules and then stored in a refrigerator at about 4°C or in a deep freezer at about -20°C after rapid freezing with dry-ice-acetone bath. Control bacteria grown in non-radioactive H medium were treated similarly.

The technique used for labeling T1 phage with high specific activity was essentially the same as that described by Stent and Fuerst (9).

The P^{32} labeled phage lysate was diluted 100 fold in cold GCA medium and stored at 4°C.

In order to check if there is any radioprotective effect of deoxygenation, anoxic ampoules sealed at ice-water temperature after two minute bubbling with nitrogen were prepared.

γ Irradiation

Overnight cultures of bacteria were harvested and then resuspended in fresh cold 4 % broth to minimize indirect radiation effects. Ampoules of broth suspensions in nitrogen and air atmosphere were placed in an ice-water bath and exposed to Cs^{137} γ-rays at a dose rate of 30 kR/hr. To make comparison with P^{32} killing in bacteria, exponential phase cultures in H medium were transferred into ice-cold GCA medium and irradiated in ice-water bath.

T1 phages (10^{10} particles/ml) suspended in 4 % broth or in other media (see Table II) were irradiated in a similar way except that a higher dose rate (11 kR/min) and a slightly higher temperature (ca. 10°C) were used.

Zonal Centrifugation of T1 DNA

Lysate of P^{32}-labeled phage was freed from bacterial debris by low speed centrifugation and then centrifuged in a Spinco L with No. 40 rotor at 25,000 rpm for 90 min and washed once with broth. The precipitate was suspended in GCA medium and stored at 4°C; half of it was used for DNA extraction and the other half for survival assay. To the former half a high titer of carrier phage was added and then diluted 100 fold in buffer of 0.01 M phosphate with 0.5 M NaCl and 0.001 M $MgCl_2$, pH 7.8. DNA was released from the phage by heating 10 min at 70°C (11).

Zonal centrifugation in a density-gradient of sucrose (5 to 20 %)

at neutral pH was performed as follows: 0.1 ml suspension of DNA was gently layered on the sucrose gradient (4.6 ml) and centrifuged at 29,500 rpm for 5 hrs at 20°C in a Spinco SW 39 rotor. The samples were fractionated by drop collection and then their radioactivities were counted by a gas-flow counter (Aloka LBC 20, Japan).

RESULTS

Sedimentation Profiles of T1 DNA Extracted from Phage Inactivated by P^{32} Decay and γ-Rays

DNA was extracted from P^{32}-labeled phages stored for various periods and its sedimentation was observed by sucrose zone centrifugation at neutral pH (Fig.1). A single sharp peak (Fig. 1-(a)) was observed for DNA extracted immediately after preparation of the labeled phage. This major peak indicates that the DNA molecules were not yet broken. As the P^{32} decay proceeded (from (b) to (d) in Fig. 1), there was a gradual decline of the major peak. This was accompanied by increased amount of radioactive material appearing in a broad slowly sedimenting peak which was presumably made up of DNA fragments resulting from double-strand scission. The ratio of broken to unbroken molecules was estimated from the ratio of the areas under the profile of the slowly sedimenting component to that of the total profile (Fig. 1). The fraction of broken DNA molecules thus estimated is equal, within experimental errors, to the fraction of phage killed by P^{32} which was determined by plaque forming ability (Table I).

Freifelder (11) has shown with an analytical ultracentrifugation method that when phage T7 particles are irradiated by X-rays in buffer, inactivation results primarily from the production of double-strand breaks in the DNA. In order to check if this is also the case of

T1 phage, the sucrose zonal centrifugation method mentioned above was used as well as the sedimenting boundary method adopted by Freifelder (11). Both methods led to the same conclusion: the fraction of broken DNA molecules is approximately equal to the fraction of killed phage particles by γ-rays in buffer. Table I includes the results obtained from zonal centrifugation.

Inactivation of T1 Phage by γ-Rays and P^{32} Decay

Fig. 2 shows the survivals of T1 phage plated on four different host strains after it was irradiated in both by γ-rays in extracellular condition. We shall define the host cell reactivable fraction (HCR fraction) by $(D_n - D_s)/D_n$. D_n and D_s are the doses required for equal phage survival plated on hcr$^+$ strains H/r30 and H/r30-R, both with normal host cell reactivation (HCR) ability, and on sensitive hcr$^-$ strains R11 and B_{s-1}, respectively. Like UV irradiated T1 phage (2, 14), the γ-rayed phage had higher plaque counts on the hcr$^+$ strains than on the hcr$^-$ strains. However the HCR fraction of γ-ray damage was much smaller than that of UV damage. It may be concluded that about 20 % of lethal damage in phage DNA produced by γ-ray irradiation in aerobic broth is reparable by the bacterial HCR mechanism effective for UV damage (see Table II). This agrees with the previous report by Sauerbier (17). However, we found that HCR fraction of γ-ray damage becomes larger when induced in anoxic condition, amounting to about 30 %. Dose modifying factor (DMF) by deoxygenation, i.e., the ratio of dose required for an equal survival in anoxic to that in aerobic condition, was about 1.20 ± 0.07 with the hcr$^+$ strains as hosts, and about 1.05 ± 0.06 with the hcr$^-$ strains.

The radiation sensitivity of T1 phage was also affected by various chemical substances added to phage suspensions at the time of irradiation. Table II summarizes the experimental data on HCR fraction and D_{37} values (dose required for 37 % survival) of T1 phage inactivated by γ-rays in various conditions. Protective ability of nutrient broth against indirect action of γ-rays seems to be of the same order as that of GCA medium, because the HCR fraction of phage damage induced by aerobic γ-irradiation is essentially constant in nutrient broth, in GCA medium with and without cysteine. It should be noted that HCR fraction becomes zero for T1 irradiated in buffer regardless of the presence of oxygen.

A "negative oxygen effect" was found for T1 phage irradiated in buffer with histidine. Histidine was presumed to be equivalent to broth in regard to the protective ability against indirect action of ionizing radiation on T-even and T7 phages (11). The negative oxygen effect found with T1 phage was eliminated by addition of cysteine to the histidine-containing buffer solutions.

The survivals of T1 phages which were inactivated *in vitro* by P^{32} decay and then assayed by plating on host strains H/r30 and B_{s-1} are shown in Fig. 3 as a function of the decayed fraction of P^{32} atoms. In contrast to UV and γ-ray treatments, the survival of P^{32}-labeled T1 was the same whether plated on H/r30 or on B_{s-1}, regardless of the presence of oxygen during the process of P^{32} decay. These results demonstrate that P^{32}-induced lethal lesions are qualitatively different from those induced by γ-rays.

Since the number of DNA-phosphorus atoms per phage is 1.4×10^5 (9), the killing efficiency per P^{32} disintegration calculated from the present experiments is 0.10. This agrees with that reported by Stent

and Fuerst (9).

Inactivation of E. coli by γ-Rays and P^{32} Decay

For stationary phase cultures, the dose effect curves of γ-rayed bacteria are shown in Fig. 4. γ-Ray sensitivities of H/r30 (H/r30-R), R11 and B_{s-1} for aerobic irradiation are approximately in the ratios 1 : 1.3 : 3.8. Fig. 4 demonstrates also the variation of anoxic protection for different strains. The DMF by deoxygenation was 1.5, 2.4 and 2.6 for B_{s-1}, R11 and H/r30, respectively.

The sensitivity difference between H/r30 and R11 seems to be solely due to the difference in their UV HCR charactor (14), i.e., the ability to excise and to repair UV DNA damage. Hence, the higher sensitivity of B_{s-1} than of R11 means that B_{s-1} has a defect in a repair mechanism other than the HCR mechanism. Mattern et al. (8) have recently proved that B_{s-1} is a double mutant defective at hcr and X-ray resistance (exr) loci.

On the base of the above-mentioned conclusions, we may take the sensitivity difference between H/r30 and R11 as the measure of the fraction of γ-ray lesions repaired by the HCR mechanism (see footnote (b), Table III). The sensitivity difference between R11 and B_{s-1} may be the measure of the fraction of lesions repaired by the "EXR" system, a repair mechanism effective for X-ray damage (see footnote (c), Table III). These estimated reparable fractions are shown in Table III with the relevant data. The HCR fraction of γ-ray lesions induced in E. coli in anoxic or aerobic broth is almost equal to the HCR fraction of lesions in T1 phage irradiated in anoxic broth (Table II). This suggests that the HCR system may repair the γ-ray damage produced on phage DNA with the same efficiency as that on bacterial DNA.

Fig. 5 shows the survival curves of P^{32}-labeled bacteria stored at 4°C under aerobic and anoxic conditions. Survivals of non-radioactive cells similarly treated remained unchanged during the storage period. The B_{s-1} was about two times more sensitive to P^{32} decay than other three strains which showed an identical sensitivity within experimental errors. It will be also seen that the labeled cells stored under aerobic condition were inactivated more rapidly than those stored under nitrogen for all the strains tested. The DMF by anoxic storage were 1.5 for B_{s-1} and 1.7 for the other strains (see Table IV).

The characteristics of P^{32} killing in bacteria compared to those of γ-ray killing are: (1) difference in P^{32} sensitivity between B_{s-1} and the resistant strains is very much reduced when compared with the difference in their γ-ray sensitivities; (2) DMF by deoxygenation is much smaller for killing of resistant strains by P^{32} than by γ-rays, while it is essentially the same for inactivation of B_{s-1} by P^{32} and by γ-rays.

Estimation of Transmutation-Induced Fraction of P^{32} Lethal Damage in Bacteria

For one hit response as shown in Fig. 5, the survival $\underline{s}$ of P^{32}-labeled bacteria is given by the formula

$$\underline{s} = 10^{-K\Delta} \qquad (1)$$

where Δ is the fraction of P^{32} atoms decayed and K is a rate constant of killing. Following Strauss (18) and Stent and Fuerst (9), we assume that the lethal lesions induced by P^{32} in bacteria can be divided into damage caused by P^{32} transmutation and that caused by β-rays. Then, neglecting the second order interaction term, we have

$$K(P^{32})_{r,s} = K(t)_{r,s} + K(i)_{r,s} \qquad (2)$$

where K(t) and K(i) are the rate constants due to P^{32} transmutation and ionization from β-rays, respectively; r and s denote γ-ray resistant strains and the sensitive mutant B_{s-1}, respectively.

For simplicity we assume that the transmutation of P^{32} has the same killing efficiency for B_{s-1} as for resistant strains:

$$K(t)_r = K(t)_s \qquad (3)$$

This assumption is valid for the case of P^{32}-labeled T1 phage which is supposed to be inactivated solely by the transmutation effect because of the negligible contribution of β-rays (see the following paragraph).

The average number of ions produced within a DNA molecule of spherical shape by a β-particle originating from an incorporated P^{32} atom is given by:

$$\frac{2}{3} R \times (LET) \times \frac{1}{\delta E} \qquad (4)$$

where 2R/3 is the mean path length of the β-particle traversing the supposed spherical DNA molecule of radius R, LET is the average linear energy transfer of P^{32} β-rays (about 2×10^6 eV·cm^2/gm in DNA, see reference (19)) and δE is the energy loss per primary ionization (70 eV). R is about 2.8×10^{-6} gm/cm^2 since T1 DNA is about 9×10^{-17} gm per particle (9). From equation (4), we obtain about 0.05 ionization per P^{32} disintegration per phage DNA. This corresponds to 3×10^3 rads per phage lethal hit by P^{32} decay. Since the γ-ray D_{37} dose for T1 phage in GCA medium is about 9×10^4 rads (Table II), the β-rays are responsible for no more than 10 % of the P^{32}-induced lethals.

The short range hit associated with P^{32} induced free radicals, which was recently proposed by Harriman and Stent (20) to account for about half of the P^{32} lethal actions on T4 phage, does not appear to be important for T1 phage. This is probably because of the different

inactivation conditions: the storage medium used for T4 phage, i.e., the buffered saline fortified with 0.17 % peptone, is about two times less protective against indirect action of ionizing radiation than the GCA medium used for T1 phage (see Table II).

Finally, we can derive from equation (2) and (3),

$$\frac{K(P^{32})_s}{K(P^{32})_r} = \frac{K(t)_s + K(i)_s}{K(t)_r + K(i)_r} = \frac{1 + \left[K(i)_s/K(t)_r\right]}{1 + \left[K(i)_r/K(t)_r\right]} \qquad (5)$$

The ratio of $K(i)_s$ to $K(i)_r$ can be approximated by the ratio of $K(\gamma)_s$ to $K(\gamma)_r$, where $K(\gamma)_s$ and $K(\gamma)_r$ are rate constants for killing by Cs^{137} γ-rays in B_{s-1} and that in H/r30, respectively. Using the numerical values given in Tables III and IV, we find that K(i)/K(t) is 0.43 and 1.7 for strain H/r30 and B_{s-1}, respectively. The contribution of transmutation in P^{32} killing is about 40 % for B_{s-1} and 70 % for H/r30, the rest being due to β-rays.

Assuming that the lethal lesion due to P^{32} transmutation is independent of oxygen, we can also calculate the transmutation-induced fraction from the data of anoxic protection against cell killing by P^{32} decay and by γ-rays (Tables III and IV). This method of calculation yields that; transmutations account for 25 and 40 % of P^{32} killing in B_{s-1} and H/r30 strains, respectively. However, this may be rather a crude estimation, because oxygen effect is complicated even in T1 phage.

DISCUSSION AND CONCLUSIONS

P^{32} Effects on T1 Phage

Ultracentrifugation analysis of P^{32}-labeled T1 DNA led to the conclusion that the fraction of phage killed by P^{32} decay is approximately equal to the fraction of broken DNA molecules by double-strand scission.

The double-strand breaks should be primarily caused by P^{32} transmutation. The β-ray effect is negligible (see the aforegoing sections of this paper). Steinberg and von Borstel (21) have also shown this experimentally (see the last section of this Discussion). Thus, the present results strongly support the hypothesis proposed by Stent and Fuerst (9) that inactivation by P^{32} incorporated into phage DNA is due to a complete cut of DNA double helix due to P^{32} transmutation.

Every P^{32} decay in DNA is supposed to produce one single-strand break (10). Since the killing efficiency (i.e., double-strand break efficiency) of T1 phage is about 0.1, one out of ten single-strand breaks apparently produces one double-strand break, whereas all single-strand breaks seem to have no lethal effect. This may mean that the single-strand breaks in phage DNA are repaired. Very recently, Ogawa and Tomizawa (22) have shown that rejoining of P^{32}-induced breaks in lambda phage DNA do in fact occur in the host cells of normal as well as in the recombination-deficient strain of *E. coli* K-12.

γ-Ray Effects on T1 Phage

The strains H/r30 and H/r30-R possess the normal efficient repair mechanism for UV lesions produced in their DNA and in DNA of T1 phage, but *hcr*⁻ mutants R11 and B_{s-1} are defective in this repair system (1, 2, 14). The HCR system can also reactivate, though to a less extent, γ-ray lesions of bacteria as well as those of free T1 phage irradiated in broth (Tables II and III).

This repair system was quite ineffective in repairing lethal lesions produced in T1 phage *in vitro* by P^{32} transmutation or by γ-ray irradiation in buffer. For the simplest explanation it may be assumed that the HCR system cannot reactivate DNA double-strand scission and

that γ-ray irradiation of T1 phage in broth produces lesions other than double-strand breaks. Freifelder (11) showed by ultracentrifugation analysis of the DNA of X-irradiated free T7 phage that after irradiation in broth only about 40 % of the phage are inactivated by double-strand breaks and the rest by other lesions, presumably by pyrimidine damages. The present experiments with T1 phage were not designed to answer the question if pyrimidine damages are involved in the reparable fraction of γ-ray lesions.

P^{32} Effects on E. coli Cells

In a previous paper (23), we reported that the mutation induced in the silkworm by the decay of P^{32} incorporated into gonads is due to the effect of P^{32} β-rays and that the transmutation effects are negligible. This result indicated that β emission might contribute more than transmutation to P^{32} inactivation even for bacteria. A similar possibility has been already voiced by several workers (9, 18, 24) on the basis of other reasonings. It is plausible to assume that in E. coli, the β-rays originating from P^{32} incorporated into RNA and other cytoplasmic constituents play an important role in killing the cells. Because the present study revealed that the amount of P^{32} incorporated into RNA was about five times as large as that incorporated into DNA.

It is concluded from the present experiments that in B_{s-1} strain only 40 % of the P^{32} killing is accounted for by P^{32} transmutation, and 60 % is due to β-ray effect. In H/r30, because of its repair ability of the β-ray damage, 70 % of P^{32} killing is due to transmutation and the residual 30 % to β-ray effect. This result obtained with H/r30 strain is in good agreement with that deduced by Ishiwa et al. (25): About 35 % are contributed by β-rays in the P^{32} killing of Salmonella.

This organism is believed to have repair mechanism for β-ray effects.

In contrast to the present conclusion, Apelgot and Latarjet (26) have recently proposed that the lethal effects of P^{32} and P^{33} are not due to the effect of β-rays or recoil nucleus but they are due to the transmutation event itself. This was concluded by an experimental result that in E. coli B/r killing efficiency of P^{32} decay was the same as that of P^{33} (with maximum β energy of 0.25 MeV and recoil energy of 5.1 eV). However, most of P^{33}-induced lethal lesions may be due to β-ray effect. A calculation based on Loevinger's equation (19) shows that the absorbed dose of β-rays in a small sphere, such as E. coli nucleus uniformly labeled by P^{33}, may be three to four times as large as that labeled by P^{32} as a consequence of several times larger LET of P^{33} β-particle than that of P^{32}. Moreover, Steinberg and von Borstel (21) have recently shown that the killing efficiency of P^{33} in phage T4 was about one-fifth of that of P^{32}. This indicates that inactivation is caused by the recoil of the sulfur atom accompanying the transmutation.

Hence we may conclude that the lethal effect of P^{32} transmutation is predominant only in small viruses, whereas it becomes negligible in higher organisms because of the increased effect of β-rays. The bacteria are intermediate in the sense that the amount contributed by transmutation is of the the same order as that by β-rays.

Our conclusion is based on the assumption that the killing efficiency of P^{32} transmutation is not affected either by the HCR system or by the EXR system (see equation (3)). The question remains whether or not this assumption is valid. The lethal damage produced by P^{32} transmutation has to be attributed to ~~be~~ double-strand breaks of DNA. Single-strand breaks would be reparable or non-lethal as indicated by P^{32} inactivation of T1

phage infecting these E. coli strains. McGrath and Williams (6) and Kaplan (7) reported that E. coli B/r (hcr$^+$, exr$^+$) can restitute X-ray induced single-strand breaks, but not double-strand breaks which lead to loss of viability in this strain. We observed that strain B_{s-1} lacking both HCR and EXR repair abilities was found to be only slightly more sensitive than H/r30 to killing by heavy charged particles from $B^{10}(n, \alpha)Li^7$ nuclear reactions[3]. This makes marked contrast to the case of γ-ray inactivation (Fig. 4). Furthermore, it has been reported that B_{s-1} and B/r were equally sensitive to inactivation by accelerated Neon nuclei (27). These data support the view that the EXR repair system cannot reactivate the severe DNA damage produced by densely ionizing radiation, which could include double-strand breaks (28).

However, evidence is still lacking to decide whether or not the EXR repair system can reactivate double-strand breaks induced by P^{32} transmutation in bacterial DNA. Hence our estimates are upper limit values of β-ray contribution in P^{32} killing in bacteria.

ACKNOWLEDGMENTS

The author wishes to express his deep appreciation to Dr. Sohei Kondo of the Department of Fundamental Radiology, Osaka University, for his valuable suggestions and criticisms throughout the course of the experiment and for many helpful comments during the preparation of this manuscript. The author wishes to express his deep gratitude to the late Dr. Seiji Matsumura for his continuous suggestions and encouragement.

Footnote

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FIGURE LEGEND

Fig. 1. Sedimentation profiles for DNA extracted from T1 phage inactivated to various survival levels by P^{32} decay. Distribution of P^{32} activity after sucrose zonal centrifugation for 5 hrs at 29,500 rpm on Spinco model L is plotted as percentage of total radioactivity. The ratio of area under the slowly sedimenting component (hatched area with vertical lines in Fig. 1-(b)) to the total area of the whole profile gives a measure of the fraction of broken DNA molecules. The number assigned to each profile indicates percentage dead phage determined by plaque assay.

Fig. 2. Survivals of T1 phages irradiated by γ-rays in broth in the presence (A: solid symbols) or absence (N: open symbols) of oxygen and then plated on different host strains. ●, ○ *E. coli* H/r30; ■, □ H/r30-R; ◆, ◇ R11; ▲, △ B_{s-1}. All symbols represent geometrical means of three to four independent experiments.

Fig. 3. Survivals of P^{32} labeled T1 phages stored at 4°C for various days and then assayed by plating on H/r30 (○) and B_{s-1} (△). (a) Storage in air. (b) Storage in nitrogen. Each symbol corresponds to the average of four independent experiments. The specific radioactivity of P^{32} in the medium used for phage labeling was 250 mCi/mg-P.

Fig. 4. γ-Ray survival for stationary phase cultures of *E. coli* H/r30 (●, ○), H/r30-R (■, □), R11 (◆, ◇) and B_{s-1} (▲, △), under aerobic (solid symbols) and anoxic (open symbols) irradiation. Each symbol shows the geometrical mean of three independent experiments.

Fig. 5. Survival curves of P^{32} labeled E. coli cells stored under aerobic (solid symbols) and anoxic (open symbols) conditions at 4°C. The specific activity of P^{32} incorporated into the cell was 35 mCi/mg-P. Symbols correspond to the geometrical means of three independent experiments, except H/r30-R and R11, where they correspond to single experiments.

● , ○ H/r30; ■ , □ H/r30-R; ◆ , ◇ R11; ▲ , △ B_{s-1}.

Table I Correlation between fraction of broken DNA molecules and fraction of phage killed by P^{32} decay or γ-rays

Treatment	Dose	Per cent dead phage	Per cent broken DNA[a]
	(k rads)		
γ-ray irradiation in buffer[b]	7.5	32	36 ± 4[c]
	13.5	57	54 ± 7
	18.5	84	76 ± 15
	$(1-e^{-\lambda t})$[d]		
P^{32} decay	0.02[e]	0	0
	0.45[e]	20	20 ± 6[c]
	0.05[f]	38	43 ± 7
	0.09[f]	56	65 ± 9
	0.18[f]	89	>95

(a) For detail, see legend to Fig. 1.

(b) T1 phage at a titer of about 2×10^{11} particles/ml suspended in 0.01 M phosphate buffer. To this was added small amount of very lightly P^{32} labeled phage (1 mCi/mg-P). The irradiation was done in the presence of oxygen. The disagreement of phage lethal dose in this experiment with that given in Table II is due to the difference in phage concentrations.

(c) Calculated from two independent experiments.

(d) Fraction of P^{32} atoms decayed.

(e) Lightly P^{32} labeled, 10 mCi/mg-P. i.e., 5 P^{32} atoms per phage DNA.

(f) Heavily P^{32} labeled, 150 mCi/mg-P. i.e., 75 P^{32} atoms per phage DNA.

Table II Variation of D_{37} and "HCR" fraction of phage T1 irradiated by γ-rays in different media in the presence or absence of oxygen

Irradiation condtion		D_{37} of T1 phage plated on different hosts (k rads)		"HCR" fraction[a] (%)	DMF by deoxygenation[b]	
Media[c]	Atmosphere	H/r30($\underline{hcr}^+$)	B_{s-1}($\underline{hcr}^-$)		H/r30	B_{s-1}
Nutrient broth(N.B.)	Air	117 ± 4	95 ± 2	19	1.2_0	1.0_5
	N_2	140 ± 6	100 ± 4	29		
N.B. + cysteine	Air	195 ± 13	165 ± 14	15	1.2_3	1.1_5
	N_2	240 ± 11	190 ± 16	21		
GCA medium	Air	95 ± 6	79 ± 5	17	1.1_6	1.0_4
	N_2	110 ± 7	82 ± 4	25		
GCA medium + cysteine	Air	165 ± 8	140 ± 10	15	1.2_7	1.1_4
	N_2	210 ± 16	160 ± 9	24		
Peptone[d]	Air	44 ± 7	44 ± 9	0	-	-
Histidine + cysteine in buffer[e]	Air	160 ± 15	138 ± 12	14	1.2_1	1.2_3
	N_2	193 ± 20	170 ± 15	12		
Cysteine in buffer	Air	158 ± 10	132 ± 9	16	1.2_0	1.2_1
	N_2	190 ± 14	160 ± 11	16		
Histidine in buffer	Air[f]	71 ± 10	68 ± 7	4	0.24	0.23
	N_2	16 ± 3	16 ± 2	0		
Buffer only	Air[f]	4.0 ± 0.7	4.0 ± 0.6	0	1.1_5	1.1_5
	N_2	4.6 ± 0.4	4.6 ± 0.4	0		

(a) $\left[D_{37}(\underline{hcr}^+) - D_{37}(\underline{hcr}^-)\right] / D_{37}(\underline{hcr}^+)$

(b) $D_{37}(N_2) / D_{37}(Air)$

(c) Concentrations of substances used: N.B.; 4%, cysteine; 10^{-2} M, histidine; 10^{-3} M.

(d) Buffered saline plus 1.7 g/l of peptone (see reference (20)).

(e) 10^{-2} M phosphate buffer, pH 7.8.

(f) Survival curves were not exponential.

Table III Two types of reparable lethal γ-ray lesions according to relative sensitivities of E. coli strains with different repair abilities

Conditions for irradiation[a]				D_{37} (k rad)			Reparable fraction (%)		
				Strains of E. coli					
Growth medium	Stage of growth	Irradiation medium	Atomosphere	H/r30 (H/r30R)	R11	B_{s-1}	HCR[b]	EXR[c]	Total[d]
Nutrient broth	Stationary phase	Nutrient broth	Air	7.5	5.7	2.0	24	49	73
			N_2	19.5	13.8[e]	3.0	29	56	85
			(DMF)[f]	(2.6)	(2.4)	(1.5)			
H medium	Exponential phase	GCA medium	Air	8.4	7.3	2.1	13	62	75
			N_2	25.0	21.0[e]	4.0	16	68	84
			(DMF)[f]	(3.0)	(2.9)	(1.9)			

(a) γ-Ray irradiation was carried out at 1 - 4°C with dose rate of 30 k R/hr.

(b) $[D_{37}(H/r30) - D_{37}(R11)] / D_{37}(H/r30)$.

(c) $[D_{37}(R11) - D_{37}(B_{s-1})] / D_{37}(H/r30)$.

(d) $[D_{37}(H/r30) - D_{37}(B_{s-1})] / D_{37}(H/r30)$.

(e) Survival curve is not exponential.

(f) Dose modifying factor by nitrogen gas treatment.

Table IV Relative P^{32} sensitivities of *E. coli* strains with different repair abilities

Specific activity (mCi/mg-P)	Storage temperature	Bacterial strain	Storage atmosphere	Slope of survival curve K(a)	Relative sensitivity to P^{32} decay(b)	Killing efficiency per P^{32} decay(c)	Dose modifying factor by deoxygenation
35	4°C	H/r30-R, H/r30, R11 (d)	Air	26.1	1.0	0.030	1.7
			N_2	15.2	0.58	0.017	
		B_{s-1}	Air	50.0	1.9	0.057	1.5
			N_2	33.3	1.3	0.038	
20	-20°C	H/r30	Air	9.3	1.0	0.018	
		B_{s-1}		24.6	2.6	0.049	

(a) $K = -(\log_{10} \underline{S})/\Delta$ (see equation 1).

(b) Relative ratio of the slope, K.

(c) The amounts of DNA were determined by the Schmidt-Thannhauser method. They were identical, 1.1×10^{-14} gm per cell in exponential phase culture for the four strains used.

(d) These three strains showed identical sensitivity to P^{32} decay.

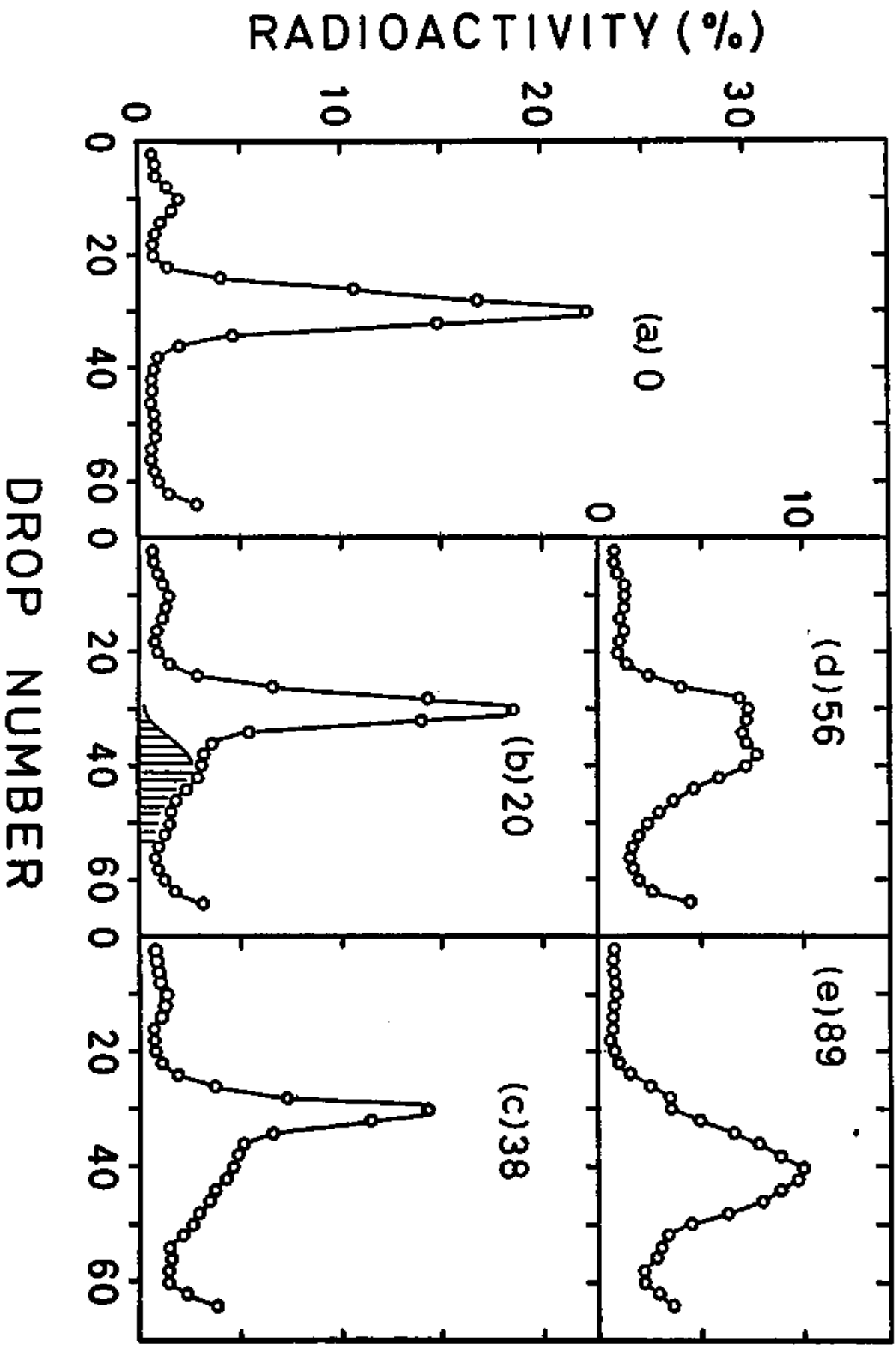
RADIOACTIVITY (%)
0
10
20
30
DROP NUMBER
0
20
40
60
(a) 0
(b) 20
(c) 38
(d) 56
(e) 89

Fig. 1

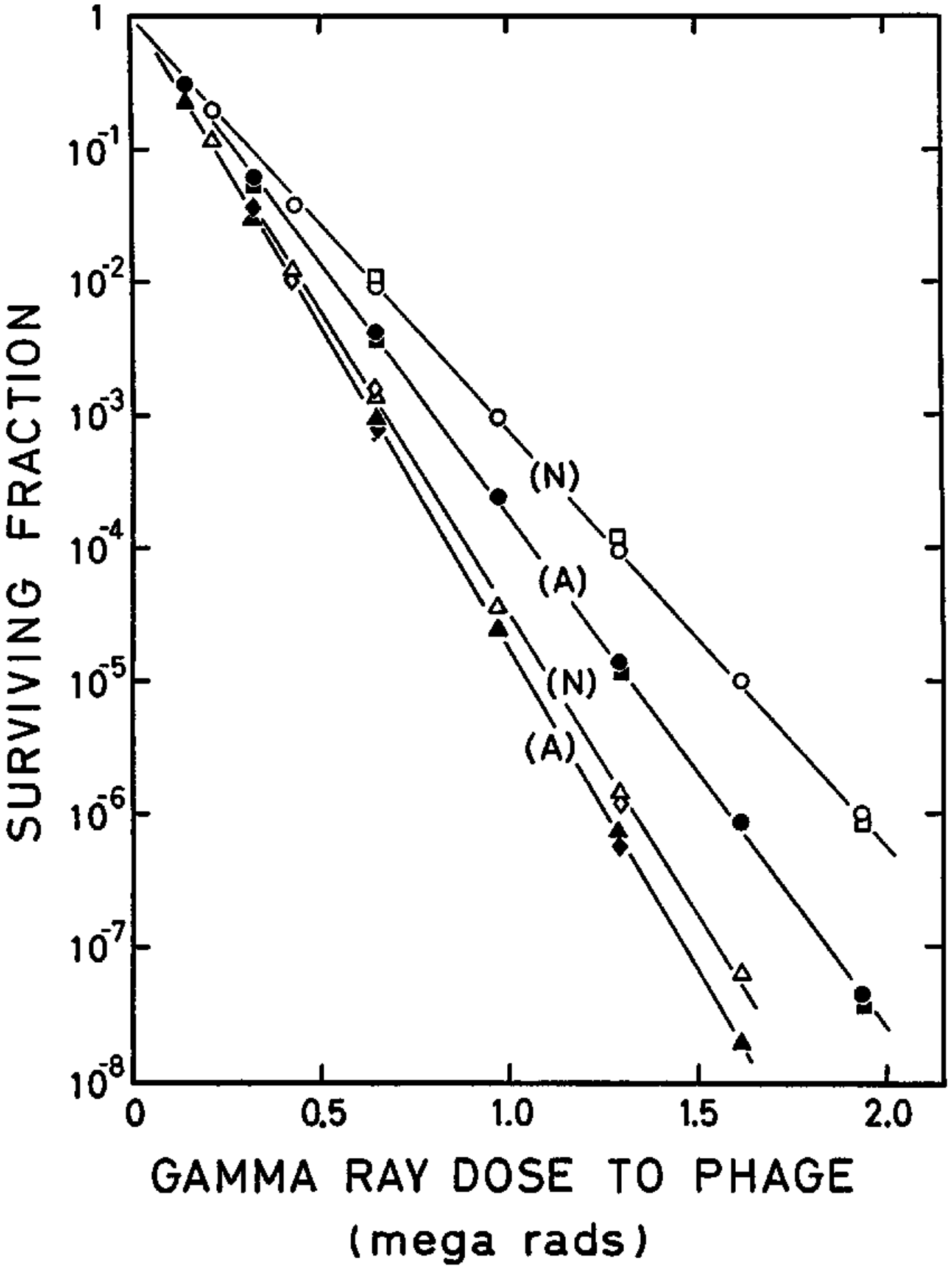
SURVIVING FRACTION
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
0
0.5
1.0
1.5
2.0
(N)
(A)
(N)
(A)
GAMMA RAY DOSE TO PHAGE
(mega rads)

Fig. 2

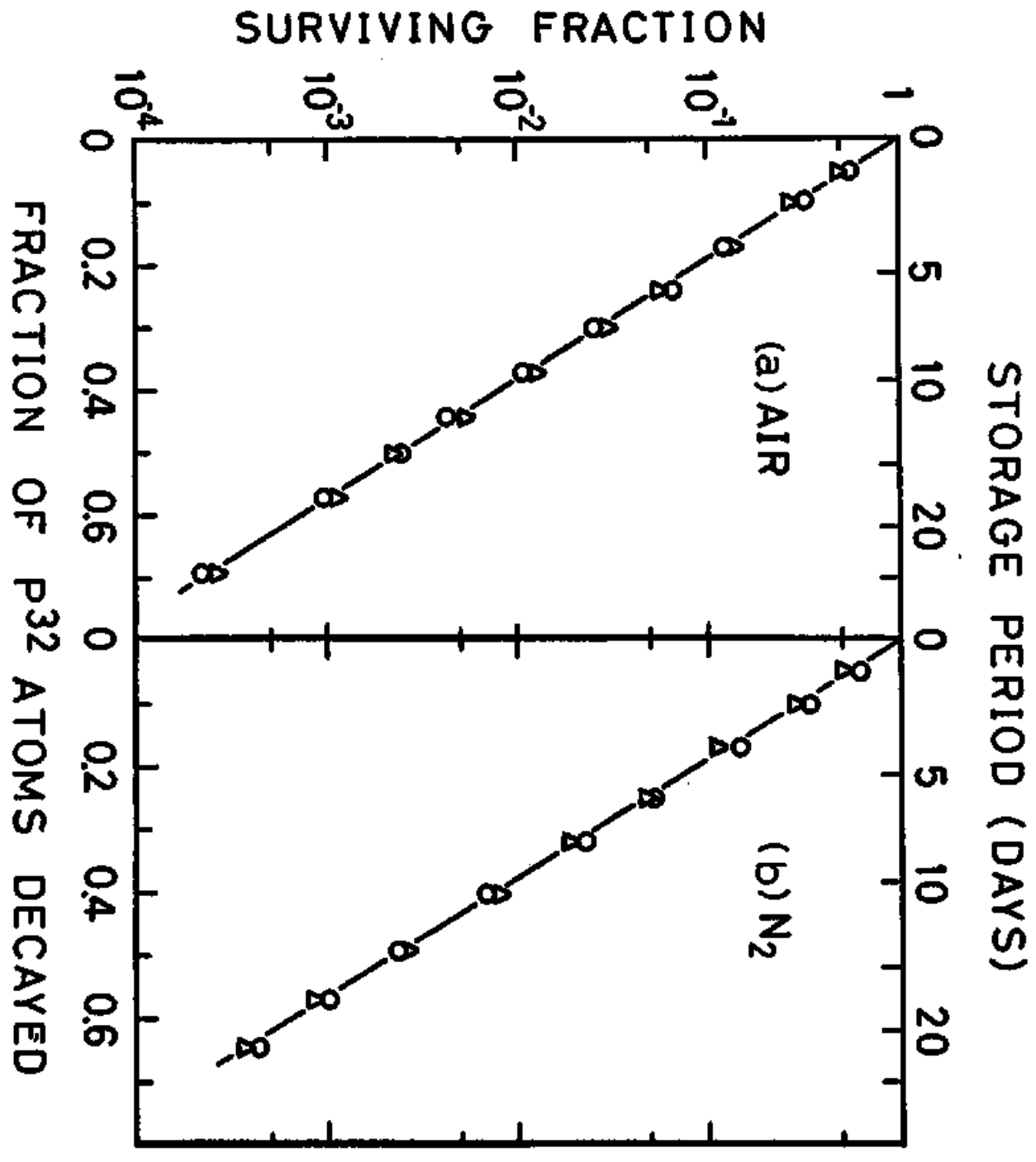
SURVIVING FRACTION
10^{-4}
10^{-3}
10^{-2}
10^{-1}
1
0
0.2
0.4
0.6
FRACTION OF P^{32} ATOMS DECAYED
(a) AIR
(b) N_2
0
5
10
20
STORAGE PERIOD (DAYS)

Fig. 3

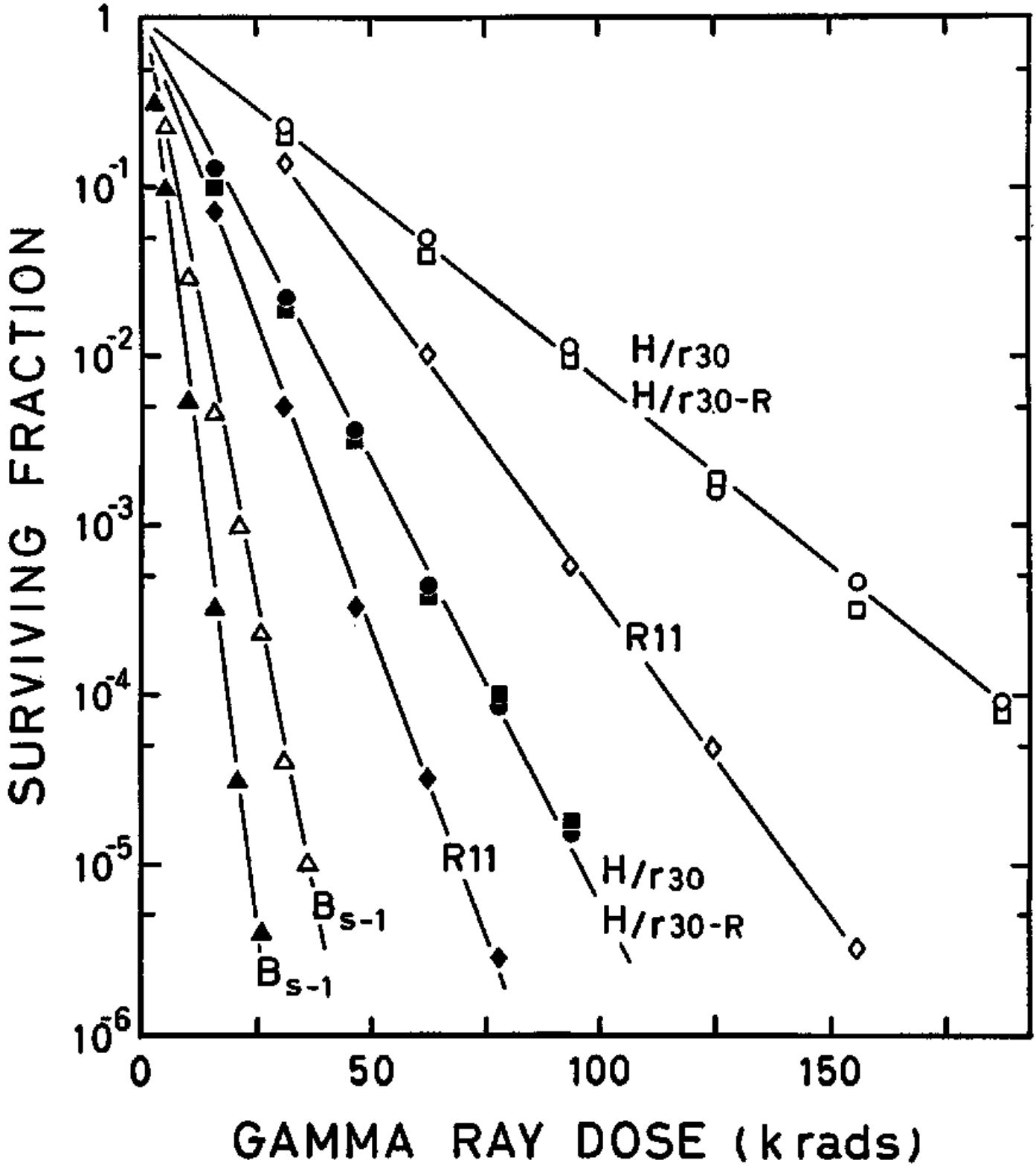
SURVIVING FRACTION
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
0
50
100
150
GAMMA RAY DOSE (krads)
H/r30
H/r30-R
R11
H/r30
H/r30-R
R11
B_{s-1}
B_{s-1}

Fig. 4

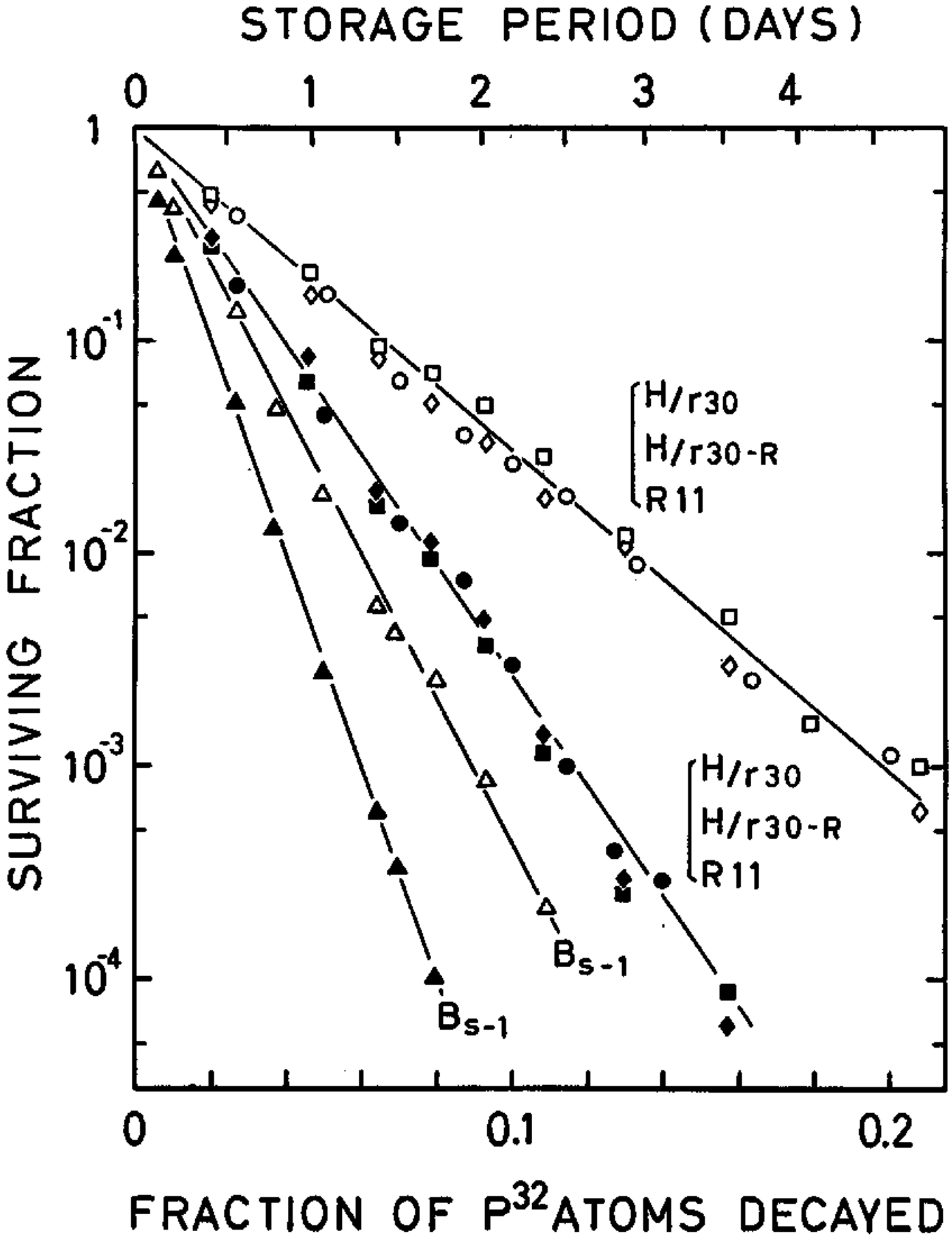
STORAGE PERIOD (DAYS)
0
1
2
3
4
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
SURVIVING FRACTION
H/r30
H/r30-R
R11
H/r30
H/r30-R
R11
B_{s-1}
B_{s-1}
0
0.1
0.2
FRACTION OF P^{32} ATOMS DECAYED

Fig. 5