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RADIATION LETHALITY IN ESCHERICHIA COLI

by

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DISCLAIMER

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ABSTRACT

Survival, post-irradiation DNA degradation, and ability of hosts to excise pyrimidine dimers from phage T7 as a function of dose have been studied in four strains of the B series of *E. coli*. It is found that the contribution to radioresistance of the three controlling loci by which the strains differ are additive both at the gross survival and dimer excision levels. The ability of hosts to excise dimers is not destroyed as a function of dose, indicating that ultimate lethality in this series of bacteria is due to a presentation of more damage to the cell than its repair system can restore. The implications of this are discussed.

INTRODUCTION

A myraid of effects have been observed following exposure of biological materials to both ionizing (X-and γ-rays) and exciting (ultraviolet) radiations. Since the advent of "Target Theory" (review: Lea, 1946), a large number of workers studied effects such as electrolyte leakage (Merrick and Bruce, 1965), disruption of protein and RNA synthesis (Pollard and Davis , 1970; Kitayama and Matsuyama, 1970), enzyme release from subcellular organelles (review: Bridges, 1969), interference with energy metabolism (Mitchell and Marrian, 1965; Bruce and Parker, 1972) in an attempt to localize the "target" molecules.

Although all types of damage, undoubtedly play a part in determining the ultimate fate of the irradiated cell, it appears that damage to DNA is of the greatest biological consequence (review: Haynes, 1964, 1966). Several different lines of experimentation have been employed to reach this concensus: DNA synthesis is the most UV sensitive process (kelner, 1953); relation of X-ray sensitivity to total DNA content (Kaplan and Moses, 1964; Freedman and Bruce, 1971); suicide experiments (Apelgot, 1965; Apelgot and Duquesue; Stent and Fuerst, 1965); electron beam penetration studies (Davis and Hutchinson, 1952), among others. Despite the fact that the above evidence is circumstantial in nature, the early finding by Hollaender and Emmons (1941) that the UV action spectrum for mutagenesis and inactivation coincides with the absorption spectrum for DNA, strongly implicates DNA as the principal target.

Damage

UV - The effects of UV on the physical properties of DNA have

been reviewed by Marmur et al, (1961) and J.K. Setlow (1965). At high doses, out of the range of biological interest, single strand breaks, DNA-DNA crosslinks (Marmur et al, 1961) and pyrimidine hydration products (Pearson, et al, 1965) have been identified. The greatest breakthrough in the field of UV photobiology came with the finding of Beukers and Berends (1960). They demonstrated that following UV irradiation there was a breakage of the 5-6 double bonds of adjacent thymine residues which resulted in the formation of a cyclobutane ring, thereby linking the adjacent residues. The so-called thymine dimer production was found to be directly related to cell killing (Wacker et al, 1962). Of minimal importance to most organisms, but significant in phage systems, is UV-induced DNA-protein crosslinking (Smith 1964,1968). This seems to cause a loss in ability of phages to inject their nucleic acid.

X-ray - X or γ -ray damages lack the molecular specificity seen above in the case of UV irradiation and a considerably wider variety of effects has been observed. Specifically, these have been categorized into direct and indirect effects. Direct effects refer to primary energy deposition into the molecule of interest. Indirect effects deal with deposition of energy into secondary molecules which are thereby rendered highly reactive with other substrates. These secondary species are usually radiolysis products of water in the form of free radicals, peroxides and superoxides (review; Bacq and Alexander, 1961).

In order to distinguish between the two types of effects, a large number of experiments have been done with dry DNA irradiated in vacuo. The results show a reduction in molecular weight of the DNA to a value which is inversely proportional to dose (Hagen and Wellstein, 1965), thereby implicating strand breakage. For DNA irradiated in solution (Cox et al, 1955)

the number of double strand breaks increases as the square of the dose showing that both strands are not subject to attack by the same water radicals. DNA strand breakage *in vivo* has been shown in many systems (Freifelder, 1965, 1966; McGrath and Williams, 1966; Goldstein *et al.*, 1973). This breakage may be the result of direct scission of the phosphodiester backbone (Peacock and Preston, 1960; Freifelder and Davison, 1963) or base damage (Latarjet *et al.* 1963; Scholes *et al*, 1960).

Repair

The early finding of Hollaender and Claus (in Smith and Hanawalt, 1969) that UV irradiated fungal spores had a lower survival if plated immediately than if incubated first in water or salt solutions was the first indication that cells could recover from the damaging effects of radiation. The pronouncement of the Watson-Crick Hypothesis and the general agreement that DNA is the principal target of interest, made so-called "Liquid Holding Recovery" and repair functions in general the subject of considerable investigation especially in relation to DNA.

Microorganisms vary widely in their ability to survive similar exposures to radiation. Since cells exposed to the same doses sustain the same amount of damage (McGrath and Williams, 1966), then it is reasonable to conclude that differences in survival levels must be due to differences in repair capacity, ability to tolerate unrepaired damage, or both.

Since the characterization of "Liquid Holding Recovery", several other recovery processes have been described. That the action of these processes are partially additive (Setlow and Carrier, 1964; Pettijohn and Hanawalt, 1964; Boyce and Howard-Flanders, 1964), implies that those

systems operative under given conditions compete in part for the same damage. Moseley and Laser (1965) and Bruce (unpublished observation) have shown a commonality of UV and X-ray repair systems in M. radiodiurans and E. coli respectively. In general, three different types of repair systems have been observed in bacteria. The first, photoreactivation (PR) seems to be specific for UV damage and the others, excision repair and recombination repair are apparently operable on both types of damage.

Photoreactivation - The term "photoreactivation" is a general term which deals with an apparent reduction of UV induced damage fostered by exposure to wavelengths in the blacklight region (review, Harm et al, 1971). There are several phenomena which fall into the category of photoreactivation. Indirect photoreactivation (Jagger and Stafford, 1965) is an enhancement of dark repair (see Excision Repair Section) and hence is enzyme dependent. Direct photochemical monomerization or photoreversal (Setlow and Setlow, 1962) is a nonenzymatic process requiring short wavelengths in the UV range. The most important, widespread and efficient process, however, is photoenzymatic repair (reviews: Cook, 1970; Setlow, 1966; Rupert and Harm, 1966). In vitro experiments with crude and partially purified yeast photoreactivating enzyme (Rupert, 1962a,b) indicate that the reaction follows Michaelis-Menten kinetics and that the necessary illumination causes photolysis of the enzyme-substrate complex with simultaneous appearance of repaired DNA.

Recombination Repair - Luria (1947) observed that E. coli B infected with UV irradiated bacteriophage at a low multiplicity yielded no bursts. However, as the multiplicity of infection (MOI) was increased above one bursts were observed with a high probability. This so-called "Multiplicity Reactivation" was the first indication that recombination-like processes could be involved in UV repair.

Recent investigations concerning recombination repair in E. coli have not used strains of the B family. Since the K12 strains of E. coli are mating strains, and mating-type recombination is likely to be enzymatic in nature, mutants defective in recombination using a mating assay were isolated from K-12 (Clark and Margulies, 1965; Howard-Flanders and Boyce, 1966). Several such mutants are now known (review: Smith, 1971) and map into three loci: recA, recB, and recC (Willetts and Mount, 1969; Willetts et al, 1969; Taylor, 1970; Storm et al, 1971).

Recombination repair, as such, was first suggested by Howard-Flanders and Boyce (1964). I has been firmly established (Howard-Flanders and Boyce, 1966) that this process is separate from excision repair (see next section). Both recombination repair and excision repair are probably of equal importance (Radman et al, 1970) as far as the extent to which damage can be repaired.

Overwhelming evidence (Howard-Flanders and Boyce, 1966; Rupp et al, 1971; Smith, 1971; Rupp and Howard-Flanders, 1968) supports the idea that a sister strand exchange takes place in this mode of repair.

Excision Repair - Dark repair as it was originally known, was so named because the presence of photoreactivating light is not necessary. The first evidence that this type of repair occurs by direct excision of damage was found simultaneously by Setlow and Carrier (1964) in E. coli B/r and Boyce and Howard-Flanders (1964) in E. coli K12. It is assumed that such a mechanism requires the sequential action of several enzymes as follows:

(1) Recognition of damage - presumably due to distortions in the regularity of the double helix.

(2) Nicking (incision) of one of the strands - either directly by

exposure to ionizing radiation or by an endonuclease in the region of the pyrimidine dimer in the case of UV.

(3) Depolymerization (excision) of the region adjacent to the nick - by some exonuclease.

(4) Repolymerization of the depolymerized region by DNA polymerase or a DNA-polymerase-like enzyme using the undamaged sister strand as a template.

(5) Sealing of the remaining nick by a DNA ligase.

With the formal proposal of this model (review: Haynes, 1966; Howard-Flanders and Boyce, 1966) investigation of the several steps necessary was undertaken in several laboratories.

Recognition and Nicking - Grossman and his group (Grossman et al, 1968; Kaplan et al, 1969) have isolated an endonuclease from Micrococcus lysodeikticus which requires double-stranded, UV irradiated DNA. Its activity is linear with dose between 10^4 and 10^5 ergs/mm^2 in vitro. Measurements of the number of incisions in relation to the number of dimers present indicate that one nick per dimer occurs under these conditions. The precise role this enzyme plays in vivo is unclear (Takagi et al, 1968). Further purification of this enzyme has been accomplished (Nakayama et al, 1971), but further clarification of its in vivo action was impossible by that group. However, mutants with depressed levels of the enzyme, repair their DNA more slowly in vivo (Okubo et al, 1971). A similar enzyme has been identified as the V gene product of bacteriophage T4 (Friedberg and King, 1971; Friedberg, 1972).

Post-irradiation Degradation - Degradation of DNA following exposure to bacteria to either UV (Stuy, 1959) or ionizing radiation (Stuy, 1960)

is a long-standing observation. Correlation between survival levels and extent of DNA breakdown have been fortuitously positive (Starvric et al., 1968), or absent (Frampton and Billen, 1966), depending on the experimental conditions. Generally speaking, however, increased depolymerization is usually accompanied by increased sensitivity (Trgovcevic and Kucan, 1969; Okubo et al, 1971; Boyle et al, 1970; Town et al, 1971). In support of this idea, agents which interfere with normal DNA synthesis patterns in unirradiated cells (e.g. phenethyl alcohol, naladixic acid) have been shown to be radiosensitizing agents contributing to excessive DNA breakdown (Dreidger and Grayston, 1971 a,b).

An exonuclease activity associated with the endonuclease of M. lysodeikticus has been described (Grossman et al, 1968; Takagi et al, 1968; Kaplan et al, 1969). The enzyme requires UV irradiated, nicked DNA. Approximately 10 bases are released per nick. In E. coli, DNA polymerase I (Kornberg's enzyme) has been shown to have exonucleolytic properties (review: Kornberg, 1969). Kornberg and co-workers have demonstrated that DNA polymerase I is capable of excising thymine dimers (Kelly et al, 1969) in a 5'⟶3' direction and that double stranded DNA nicked with a 3'-hydroxy terminus can serve as a template primer for the well-known 5'⟶3' polymerization. It is unclear, however, why the 3'⟶5' exonucleolytic activity (Kornberg, 1969) is not observed for this type of damage (Kelly et al, 1970).

A mutant (polA1) has been isolated from E. coli W3110, a K-12 derivitive, which is an amber mutant of DNA polymerase I and UV sensitive (DeLucia and Cairns, 1969). PolA1 mutants have been observed to degrade considerably more DNA post-irradiation than their parent strains and the increase in UV sensitivity is due to an increase in nuclease activity (Boyle et al,

1970). Since semiconservative DNA replication is apparently normal in polA1 mutants (Okazaki et al, 1970), the function of DNA polymerase I as a repair enzyme is implicated.

Repolymerization (Replication Repair) - The fidelity of depolymerization and repolymerization is questionable at the present time. Two models have been proposed with respect to this problem. The first mechanism, the "cut and patch" mechanism (Howard-Flanders and Boyce, 1966) proposes that depolymerization and repolymerization are, in fact, separate steps. That is, the excision is completed and the resultant oligonucleotides are released from the DNA before polymerization begins. The second model, the "patch and cut" hypothesis argues that replacement occurs with a simultaneous peeling back of the damaged region (Haynes, 1966). This suggested to Strauss (1968) the possibility of a "repair complex" which could catalyze both degradation and replication repair. The involvement of DNA polymerase I seems to favor this second alternative.

Repair replication, then, appears to be a process separate from regular semiconservative DNA duplication. The findings with polA1 mutants as well as the recent observations by Werner (1971) that the two processes require different precursors, bear this out. Billen (1969), however, has shown that semiconservative duplication is initiated at sites other than the fixed replicating origin following X-irradiation of E. coli 15T⁻. In other words, both types of replication are operable post X-ray. Whether this duplication initiates at fixed "alternate sites" on the chromosome or at regions damaged by the irradiation is not known. Similar observations were not made following UV-irradiation.

Ligation - Enzymes which catalyze the covalent joining of nicked, double-stranded DNA have been isolated and purified from E. coli and phage-

It appears, therefore, that the ultimate success of excision repair depends on the ability of the ligase to displace the nuclease from the DNA. In support of this, was the isolation (Howard-Flanders and Theriot, 1966) of "reckless" mutants which excessively and irreversibly degrade their DNA. Such mutants, of course, are extremely sensitive.

Genetic Control of Excision Repair - Although some work has been done with other organisms, notably B. subtilis (Bron and Venema, 1972 a) the bulk of recent literature deals with E. coli B, K-12 and coliphages (reviews: Smith, 1971; Adler, 1966). Apart from the polA locus (above), six other loci are known to affect excision repair in the K-12 series. Adler and Hardigree (1964) found that a mutant, lon, which interferes with septum formation also shows marked UV and X-ray sensitivity. Another mutant, lex (Howard-Flanders and Boyce, 1966) appears to control the extent of DNA breakdown post irradiation. Howard-Flanders et al, (1966) described three other loci (uvrA, uvrB, uvrC) which they conclude by building double mutants, perform the same function. Earlier experiments by Emmerson and Howard-Flanders (1965) which show no difference in X-ray response of UVR^+ and UVR^- strains indicate that these uvr loci deal with endonuclease functions. The fourth locus in this series or uvrD (Ogawa et al, 1968) which functions after uvrB. In the temporary heterozygotes $uvrD^-/uvrD^+$ $uvrD^-$ is dominant. Further characterization (Shimada et al, 1968) indicates that uvrD mutants do not incise, do excise but do not repolymerize. This would implicate uvrD as an exonuclease suppressor.

Radioresistance in the B series of E. coli appears to be controlled at four loci - three structural (fil, hcr, exr) and one suppressor (sul).

The Fil Phenotype - In 1962, Rorsch et al, observed a correlation between radiosensitivity and the formation of long filaments by sensitive

cell post-irradiation. That is, the filamentation was absent in B/r and present in B. Cells with the Fil^+ phenotype are able to undergo "nuclear" division (Adler and Hardigree, 1965), however the filaments eventually lyse and die before visible colonies are apparent. That the addition of pantoyl-lactone to cells are fil^+ both prevents filamentation and raises the UV survival to the level of fil^- cells (van de Putte et al, 1963) demonstrates the connection between the two is real. That further addition of pantoyl-lactone to fil^- cells does not increase their resistance, indicates that increased concentrations do not act in a radioprotective capacity. The same pantoyl-lactone effect was observed in the K-12 lon^+/lon^- system (Adler and Hardigree, 1965). Thus it appears that Fil and Lon are indistinguishable although they map in different regions (Adler, 1966; Taylor, 1970).

Filament formation has also been observed following a variety of non-radiation treatments - notably crystal violet (Rorsch et al, 1962), chloramphenicol (Witkin, 1967 a) and high pressure (Zobell and Cobet, 1964). These and other observations led Witkin (1967 a) to note the parallels between induction of filamentation in the B series and prophage induction in K-12 lysogenic for lambda.

The Hcr Phenotype - Host cell reactivation (Hcr) was first described by Ellison et al, (1960). Their observation was that UV irradiated phages T1, T3 and T7 when plated on B had a higher survival than when plated on B_{s-1}. Unirradiated phages gave identical plaque counts on either host. The survival curves for these phages were clearly separable when assayed on B but were almost coincidental when plated on B_{s-1}. These observations led the authors to postulate that a genetic determinant which accounted for the increased sensitivity of host B_{s-1} in relation to B also participated

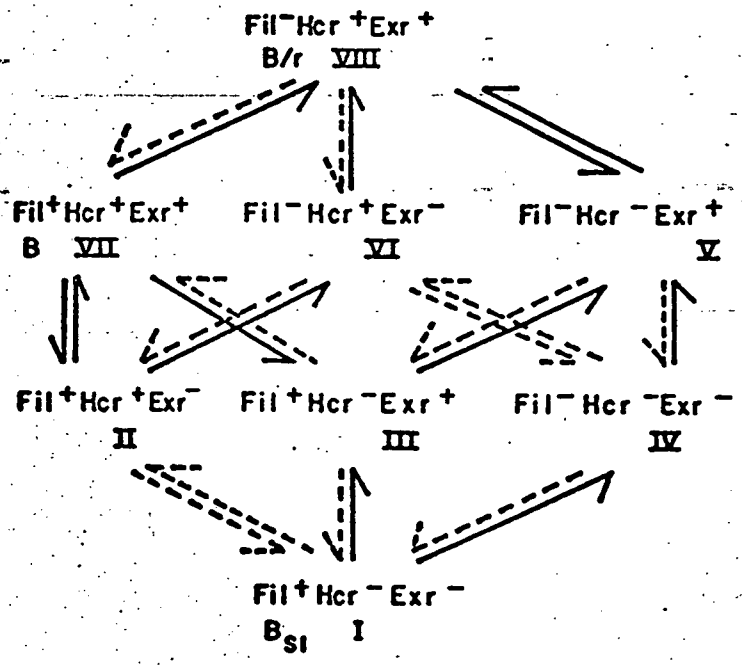
in repair of the phages. That this phenomenon was not observed following X-irradiation of T1 suggests that an endonuclease function is involved. Further studies by those authors (Hill, 1964; Feiner and Hill, 1966) confirm that reactivation is due to a host-directed function. The original investigation (Ellison et al, 1960) also indicated that T2, T4 and T5 were not subject to host cell reactivation. The identification of the V gene product of T4 as an endonuclease (Friedberg, 1972) supports the idea of hcr as an endonuclease.

The Exr Phenotype - Mattern et al (1966) have shown by transduction mapping with phage P1, that B_{s-1} is actually a double mutant of B. That is, the two functions are separable by transduction and the resultant mutant has an X-ray sensitivity intermediate between B and B_{s-1}. Witkin (1967 b) has observed that Exr^- strains regardless of their Hcr or Fil phenotypes don't produce UV induced mutations at a detectable frequency, but Exr^+ strains derived by mutation from Exr^- strains do. Exr^+ strains can produce viable DNA from DNA containing two to three times as many residual dimers as Exr^- strains. She concludes that the Exr^- phenotype restores DNA by a less efficient but more accurate method than Exr^+.

The exr locus of B maps (Mattern et al, 1966) at roughly the same position as lex in K-12 (Howard-Flanders and Boyce, 1966) and may, in fact, be the same locus.

That excision repair in the B series is controlled by 3 structural loci and that the contributions to sensitivity are additive (Mattern et al, 1966; Rorsch et al, 1962) suggested to Witkin (1967 b) that there should be four "levels" of sensitivity throughout the B series. The "level" of sensitivity, then, should depend on whether a strain in question carried 0, 1, 2, or 3 of the sensitivity loci. Since there are eight possible

Figure 1. Schematic representation of genetic control of radiation resistance in the B series of *E. coli*. Roman numerals represent the eight classes of mutants. Arrows represent possible derivations by single mutational events (From Witkin, 1967 b).


Fil⁻Hcr⁺Exr⁺
B/r VIII
Fil⁺Hcr⁺Exr⁺
B VII
Fil⁻Hcr⁺Exr⁻
VI
Fil⁻Hcr⁻Exr⁺
V
Fil⁺Hcr⁺Exr⁻
II
Fil⁺Hcr⁻Exr⁺
III
Fil⁻Hcr⁻Exr⁻
IV
Fil⁺Hcr⁻Exr⁻
B_St I

combinations of the three loci, then there should be eight classes of strains derivable from each other by single mutational events. By starting with five known genotypes and studying forward and back mutation, Witkin was able to show that all derivitives fell into one of the eight expected classes. A schematic representation is shown in Fig. 1.

Objective

It is obvious from the data presented thus far, that upon irradiation cells are faced with two opposing processes: damage and repair. The ultimate fate of irradiated cells, therefore, depends on an interplay between the two. Stated differently, the question becomes whether lethality results from a presentation to the repair system of more radiation damage than it can accomodate or whether repair activity is destroyed by radiation. What we are asking, then, is whether or not the repair system is damaged by irradiation and if so what is the contribution of the genetic pattern described by Witkin? It is to this end that the present research was undertaken.

Use of the Phage Systems

If one is to determine whether or not the repair system of E. coli is, in fact, damaged by exposure to ionizing radiation, it is necessary to present increasing doses to the repair system without creating increased damage to the substrate. Obviously then, a system must be utilized in which a fixed amount of damaged DNA is present. That is, experiments using whole cells are not suitable in that the two types of damage are not separable. Recent studies regarding the additivity of UV and X-ray exposures in E. coli (Bruce, unpublished observations) have been, at best, confusing. No apparent generalizations can be reached

from these data. Howell (1972) has studied this in whole cells of M. radiodurans with the finding that pre-treatment with doses below 600 kR followed by UV irradiation have no effect on the kinetics of dimer excision. Doses greater than 600 kR reduce the rate of excision in that organism. No conclusions, however, could be drawn with respect to destruction of "repair ability" due to increased substrate damage.

In order, then, to carry out the present studies, the repair system must be presented with an external source of DNA which contains a fixed amount of damage. There are three methods by which this may be done. The first possibility is incubation of UV irradiated DNA with a cell-free E. coli system. This has obvious limitations since breakage of the cell leads to a liberation of membrane-bound nucleases (Alper, 1968) which might not normally participate in repair.

The second alternative involves a transformation system using UV-irradiated transforming DNA. Bron and Venema (1972 a,b,c,d) have studied the properties of UV-irradiated transforming DNA extensively in B. subtilis. The only report of transformation in E. coli has been by Avadhani et al., (1969) and those data have not been verified.

The method of choice, then, is a phage system. The "T" phages have the advantage of being lytic on all strains of the B series having the desired combinations of markers. Since T2, 4, 5 and 6 carry loci which code for repair enzymes, T7 was chosen as the test organism.

MATERIALS AND METHODS

Bacterial and Viral Strains

Four strains of the B series *E. coli* were used in the course of this work. The parent strain was isolated by Bronfenbrenner (1920) and designated *E. coli* B by him. It has the genotype fil^+, exr^+, hcr^+. The radioresistant mutant, *E. coli* B/r (substrain CSH) was isolated by Witkin (1946) and carries the genotype fil^-, exr^+, hcr^+. Radiosensitive mutants B_{s-1} (originally designated B_s) (Hill, 1958) and B_{III-10} (Witkin, 1967b) have the genotypes fil^+, exr^-, hcr^- and fil^+, exr^- and hcr^+ respectively.

All strains were maintained on nutrient agar plates (see appendix) at 4°C and transferred to fresh plates monthly.

Bacteriophage T-7 (substrain 3) was generously supplied by Dr. Rosemary Elliot of Roswell Park Memorial Institute.

Culture conditions

All cells were grown at 31°C with aeration by vigorous shaking. To derive growth curves, cells were incubated in sidearm culture flasks and absorbance was determined in a Bausch and Lomb "Spectronic 20" colorimeter at 650 nm. Unlabeled *E. coli* were grown to midlog phage in either NUT broth or $M\text{-}9^+$ (see appendix) as measured by $A_{650} = 0.3$ for strains B/r (CSH), B and B_{s-1} and $A_{650} = 0.09$ for strain B_{III-10}.

For purposes of DNA labeling M-9 medium (Anderson, 1946) was supplemented with hydrolyzed casein to promote rapid growth, tritiated thymidine (NEN) and deoxyadenosine (Boyce and Setlow, 1962) to promote uptake of label (appendix). This was then designated $M\text{-}9^+$. One half generation prior to harvesting, the cultures were flooded with a 100-fold excess of cold thymidine in order to chase the activity into high molecular weight

material. At the appropriate cell concentrations (see above) cells were harvested by centrifugation and resuspended in cold M-9^{+} with the addition of cold thymidine as above.

Irradiation conditions

X-irradiation was performed at room temperature with a Westinghouse 200 kVp theraputic X-ray unit. The dose rate was 12.8 kR/min. as determined by the Ficke ferrous sulfate technique (Ficke, 1953). Cells were suspended in a cylindrical nylon irradiation chamber and agitated by forced aeration to ensure equal dose.

UV irradiation was performed using a GE germicidal lamp (85% emission guaranteed at 2537 Å) at a dose rate of either 19 ergs/mm^2-sec or 0.6 ergs/mm^2-sec. by varying source columnation. Dose rate was measured using a Jagger-type photocell (Jagger, 1961). All manipulations post-UV were performed under yellow safelights in order to avoid "phororeactivation" and "photo-reversal" of UV-induced lesions.

Phage Assays

All phage viability assays were performed with the top agar overlay method using 2½ ml nutrient top agar (see Appendix) upon plates containing nutrient agar (see Appendix). Unless otherwise stated, the indicator host was B/r (CSH).

Preparation of Phage lysates

In all cases, the parent strain (B) was used as the host. For preparation of "cold" lysates, midlog phase cultures of *E. coli* B in NUT broth were inoculated with phage suspensions at a multiplicity of infection (MOI) of 0.01. Lysates were harvested by centrifugation when the culture appeared clear to the naked eye. The resultant was decanted and stored with chloroform at 4°C until used. Before use, lysates were

warmed to room temperature.

For preparation of labeled lysates, hosts grown overnight in nutrient broth were back cultured 1:100 into M-9^{+} medium containing tritiated thymidine (20 μCi/ml). The culture was allowed to reach midlog phase and the procedure above was followed. In order to avoid loss of infectivity (Hotz et al, 1971; Cleaver et al, 1971), labeled lysates were not stored for more than 10 days.

Adsorption Kinetics

Midlog B/r cultures were infected at an MOI of 0.01. Aliquots sampled at various times post-infection were passed through membrane filters (0.45 μ pore size), suitably diluted and plated. The percentage adsorption was determined by subtracting the number of unadsorbed phages recovered by this method from the number recovered at 0 minutes post infection.

Plating Efficiency

Plating efficiency was determined using unirradiated and irradiated phages (800 ergs/mm^2). Phage suspensions were treated as above for unirradiated phages. For irradiated phages, each cell line was infected at an MOI of 0.01. After 10 minutes incubation, cultures were passed through a 0.45 μ membrane filter and the number of free phages assayed as above using B/r (CSH) as the indicator strain. This figure was then subtracted from the original titer to calculate percent adsorption. Plating efficiency is calculated using B/r as 100%.

UV Survival Curves

Hosts were grown to stationary phase in NUT broth and harvested by centrifugation. After resuspention in 0.067 M phosphate buffer, the number of cells in suspension was normalized by adjusting the A_{254} to 0.4 in a

Beckman model DB spectrophotometer. The dose rate used for strains B/r (CSH) and B was 19 erg/mm^2-sec while the dose rate used for B_{III-10} and B_{s-1} was 0.6 erg/mm^2-sec. Hosts were appropriately diluted, plated and scored as above.

Phage survival was determined by adding 0.1 ml of phage suspension, appropriately diluted in phosphate buffer, to NUT top agar seeded with B/r (CSH) as the indicator.

X-ray Survival Curves

Hosts were grown to midlog phase, harvested and resuspended as above. Suspensions were appropriately diluted in 0.067 Molar phosphate buffer and spread immediately on NUT plates. Visible colonies were scored within 18-24 hours post-irradiation.

DNA Degradation

Cells containing labeled DNA were irradiated to 40 kR. At varying times post irradiation, 0.1 ml aliquots were sampled and added to 1.0 ml ice cold 10% trichloroacetic acid (TCA). After standing one hour at ice bath temperature to solubilize low molecular weight material, samples were filtered on 0.45 μ pore size membrane filters (Millipore Filter Corp). Precipitation tubes were washed 3 times with equal volumes of ice cold TCA and added to the chimneys. The chimneys were rinsed in the ice cold TCA. Membranes were allowed to air-dry at room temperature and placed in vials for liquid scintillation counting. Ten ml of toluene based scintillation cocktail (see appendix) were added to each vial along with 1 ml of water. Vials were shaken vigorously and assayed for radioactivity in a Packard Tricarb model #3320 Liquid Scintillation Spectrometer for 10 minutes each. This method is similar to that used by Achey and Pollard (1967).

Phage Competition

Labeled E. coli were irradiated as above and infected with phage at 0 time post irradiation to an MOI of 1.0. In order to prevent the burst, chloramphenicol was added to the suspension at 8 minutes post infection to a final concentration of 25 μg/ml. Sampling and preparation proceeded as above.

Dimer Assay

Ten ml midlog cultures of hosts irradiated to 0, 10, 20, 40 or 80 kR were infected with labeled phages and incubated as above (see "Phage Competition") for one hour. To the 10 ml culture 2 ml containing 5 M $NaClO_4$ and 50 mg SLS was added (Marmur, 1961) for deproteination. Following chloroform/isoamyl alcohol extraction and ethanol precipitation, the resultant precipitate was harvested by centrifugation, washed in 0.5 M perchloric acid (PCA) and resuspended to 2.0 ml in 70% PCA. The suspension was hydrolyzed for 3 hours at 100°C (Gunther & Prusoff, 1967) and allowed to cool to room temperature. A drop of bromthymol blue in 0.05 M NaOH (Lange, 1946) was added to the hydrolysate and the solution was neutralized with NH_4OH (Sekiguichi et al, 1970).

A 10 μl aliquot of the neutralized hydrolysate was counted for radioactivity in order to determine the specific activity. Approximately 50,000 cpm (Carrier and Setlow, 1970) was applied to Dowex 1 x 8 (formate form) ion exchange columns (Sekiguichi et al, 1970). The columns (1 x 10 cm) which had been equilibrated with 0.02 M NH_4OH were eluted with 0.02 M NH_4OH, 0.2 M NH_4COOH (pH 8.8) and 0.02 M HCOOH successively. Fractions were evaporated to dryness by mild heating (50-60°C). Ten ml scintillation cocktail (see appendix) and 1 ml water were added to each vial and the vials counted for radioactivity.

Percent of total radioactivity recovered as dimers was calculated by summation of the counts in the peak fractions in the dimer region (Fig. 11) and dividing this by the sum of the counts in the peak fractions in the monomer region plus the sum of the dimer region (i.e. Dimer Peak/Dimer Peak + Monomer Peak).

RESULTS

X-ray Survival Curves

X-ray survival curves for the four strains used are presented in Figure 2. The order of resistance is B/r $>$ B $>$ B_{III-10} $>$ B_{s-1}. This is in accordance with the hypothesis of Witkin (1967b). It is difficult to compare these data with published data since the present experiments were performed using midlog phase cells. Furthermore, X-ray survival data for B_{III-10} have not been published. Goldstein et al, (1973) have determined that the survival of B_{III-10} is intermediate between that of B and B_{s-1} using stationary phase cells. This plus the observation of Town et al (1971) that log phase cells are more sensitive than stationary cells implies that the results are in good agreement.

UV Survival Curves

In order to determine whether the strains used were genotypically true, UV survival curves were generated. The results are shown in Figures 3 and 4. These curves are in reasonably good agreement with those derived by Witkin (1967b). The small discrepancies may be due to differences in experimental technique.

An interesting observation was that many of the colonies of the fil^+ strains (B_{s-1}, B_{III-10}, B) which had received low doses exhibited a sectoring which appeared as a clear, circular area. On further incubation, the ratio of clear area to clone increased. To test whether this morphology was similar to a phage-infected colony situation hosts which had not been irradiated were mixed with T7 phages at an MOI of 1.0 and immediately spread on plates. Although the frequency of sectored colonies

Figure 2. X-ray survival of midlog phase E. coli

● = B/r (CSH)

■ = B

◆ = B_{III-10}

▲ = B_{s-1}

Dose rate = 12.8 kR/min.

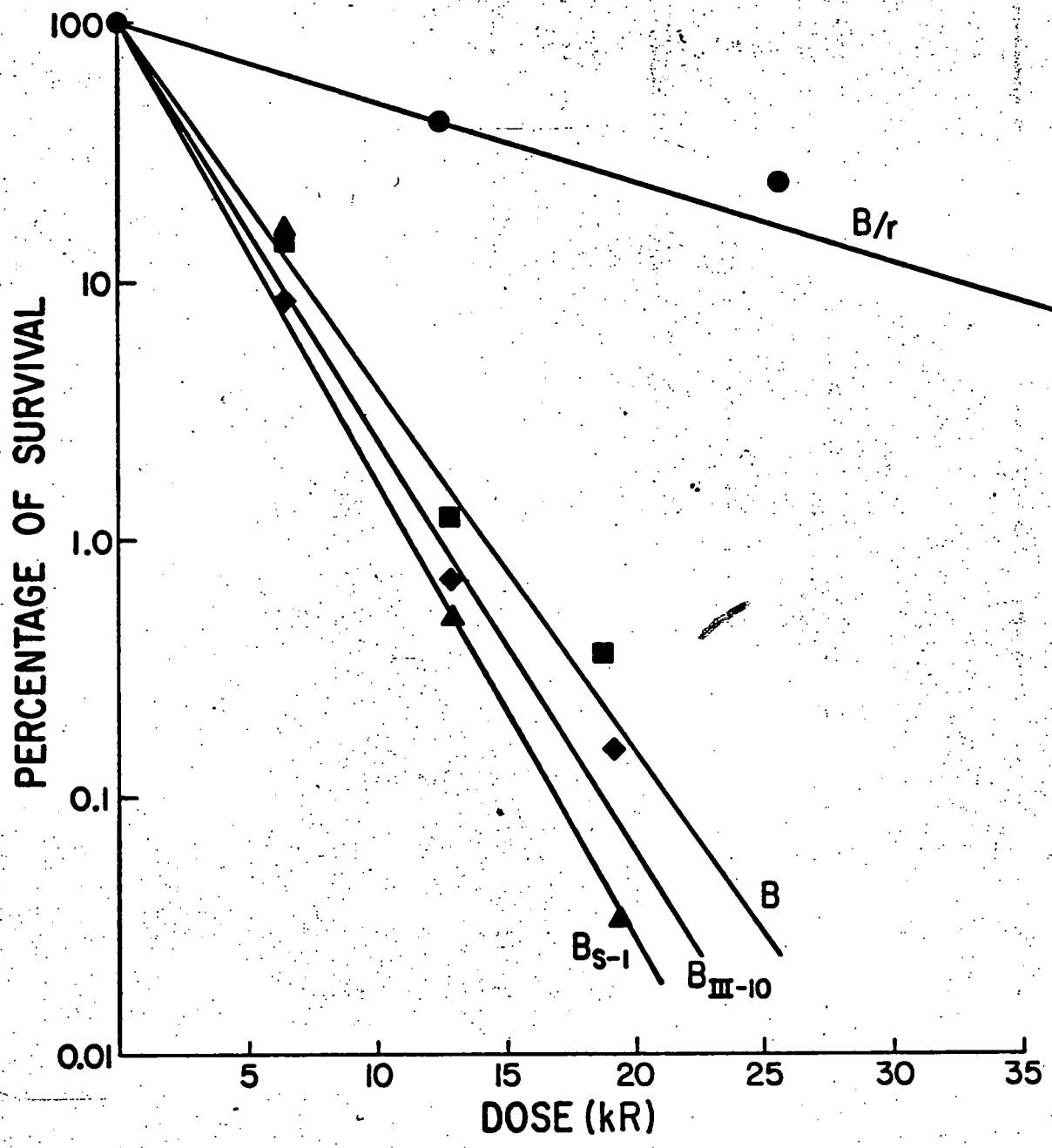

100
10
1.0
0.1
0.01
PERCENTAGE OF SURVIVAL
5
10
15
20
25
30
35
DOSE (kR)
B/r
B
B_{S-1}
B_{III-10}

Figure 3. UV survival of *E. coli* B_{s-1} and *E. coli* B_{III-10}

▲ = B_{s-1}

◆ = B_{III-10}

Dose rate = 0.6 ergs/mm^2-sec

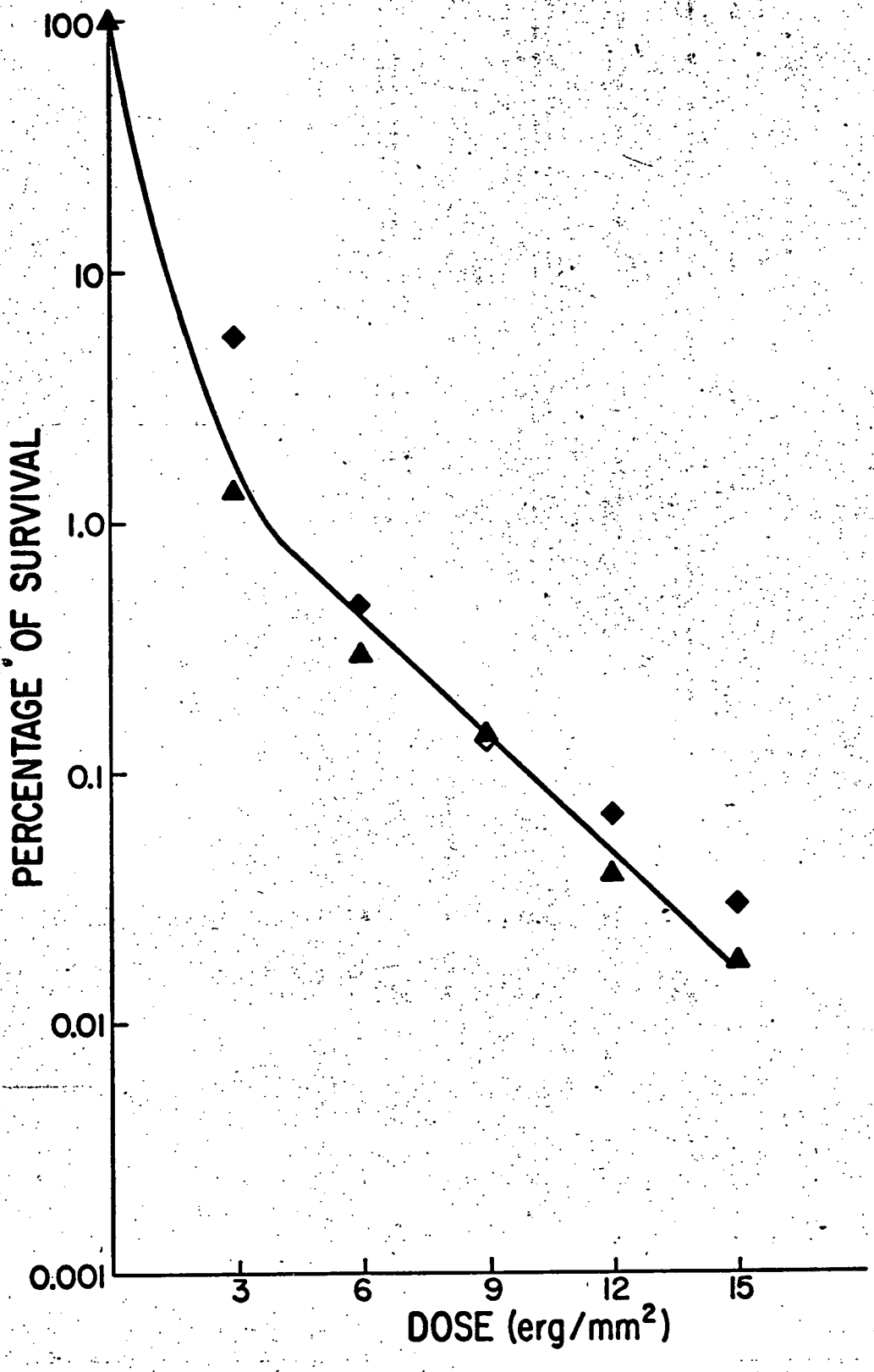
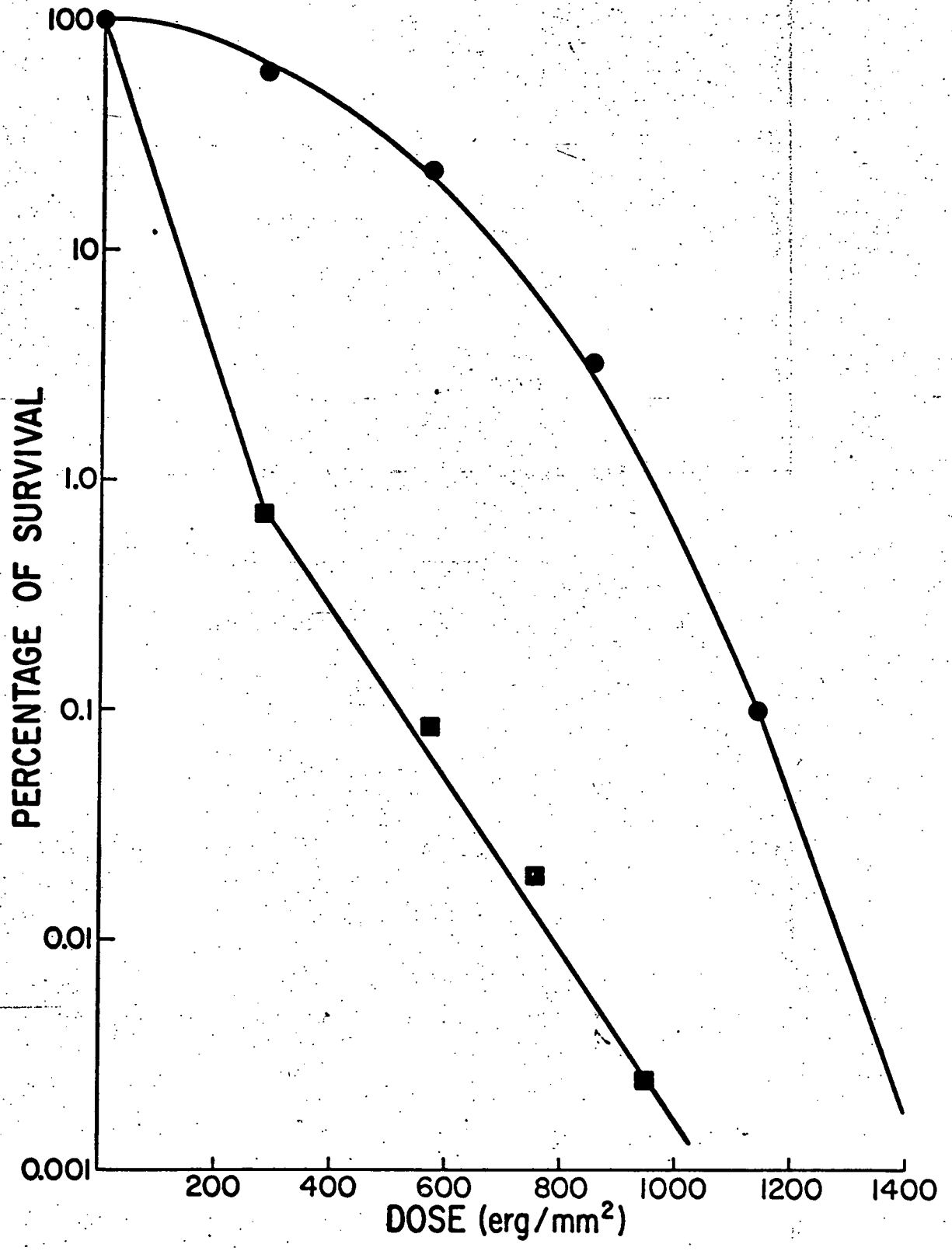

100
10
1.0
0.1
0.01
0.001
PERCENTAGE OF SURVIVAL
3
6
9
12
15
DOSE (erg/mm²)

Figure 4. UV survival of E. coli B/r (CSH) and E. coli B

● = B/r (CSH)

■ = B

Dose rate = 19 ergs/mm^2-sec.


100
10
1.0
0.1
0.01
0.001
PERCENTAGE OF SURVIVAL
200
400
600
800
1000
1200
1400
DOSE (erg/mm²)

was considerably lower than above, the morphology was strikingly similar to that observed with UV irradiated cultures.

UV Survival of T7

Recent evidence (Studier, personal communication) indicates that T7 has very pronounced endonuclease activity and that during normal infection 85% of the bases in the T7 progeny are taken from the host genome. Since we wish to study the effect of host genotypes, it is necessary to establish that phage-directed endonuclease does not participate under these experimental conditions. If UV and X-ray survival curves are similar, then the nonparticipation of UV endonuclease is indicated. This is due to the single strand breaks introduced by ionizing reduction. The results of UV survival experiments are shown in Fig. 5. These results are similar to the X-ray survival results obtained by Gampel-Jobbagy et al, (1972).

Further evidence for the nonparticipation of phage directed endonuclease was reported by Ellison et al, (1960). Then demonstrated that the effects of the Hcr phenotype are observable only in cases where phage-directed repair enzymes are inoperable or absent. Those authors were able to observe host cell reactivation with T7.

Adsorption Kinetics

Since the latent period in the B-T7 system is 20-25 minutes (unpublished observation), and the experiments to be performed later have a 60-90 minute duration, reinfection which results from the completion of the phage growth cycle must be prevented. Tomizawa and Sunakawa (1956) observed that the burst could be absorted by treatment with chloramphenicol which blocks phage-directed enzyme synthesis (Takagi et al, 1968). Since a lysed culture becomes progressively less turbid as lysis becomes more

Figure 5. UV survival of Bacteriophage T7. Dose rate = 19 ergs/mm^2-sec.

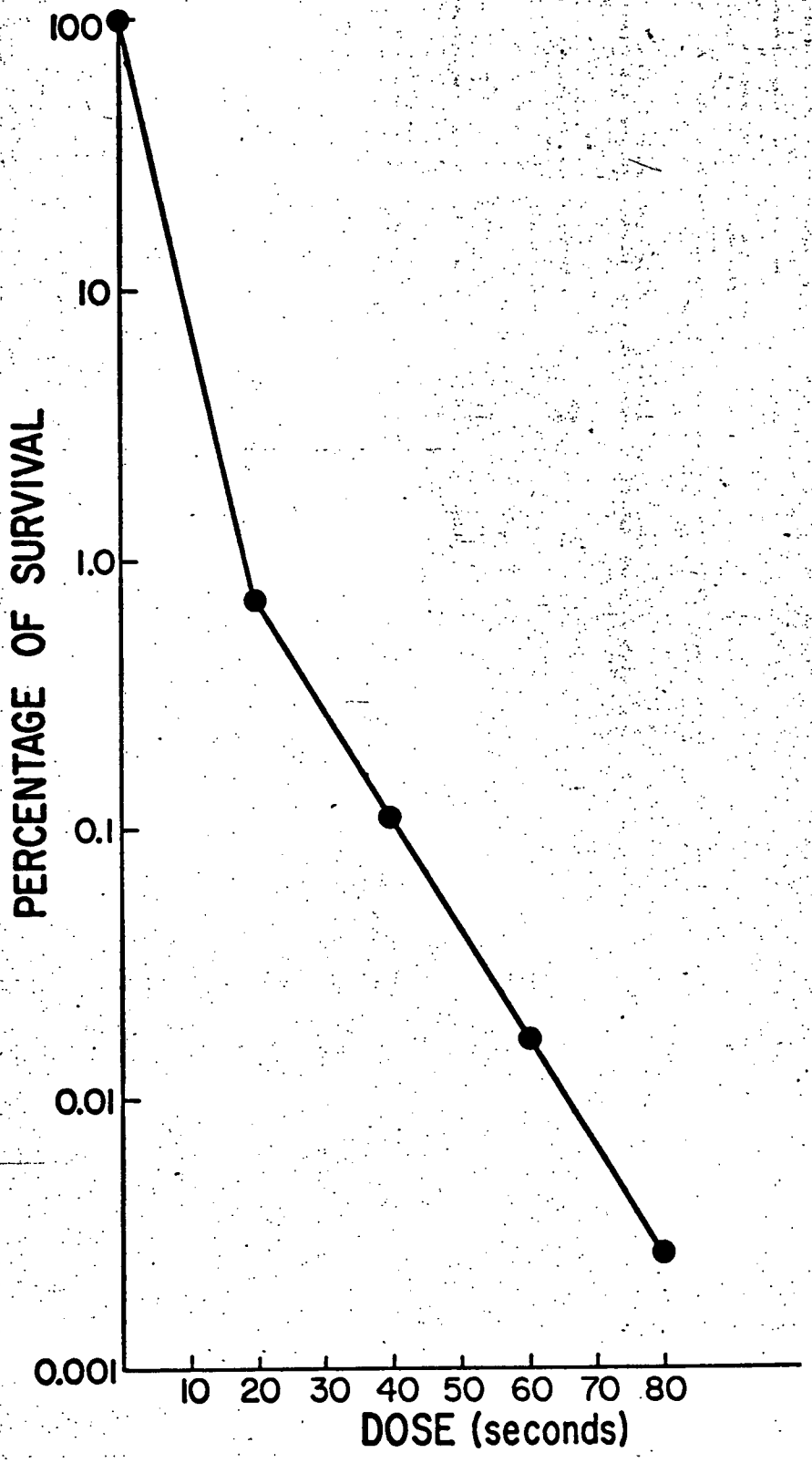
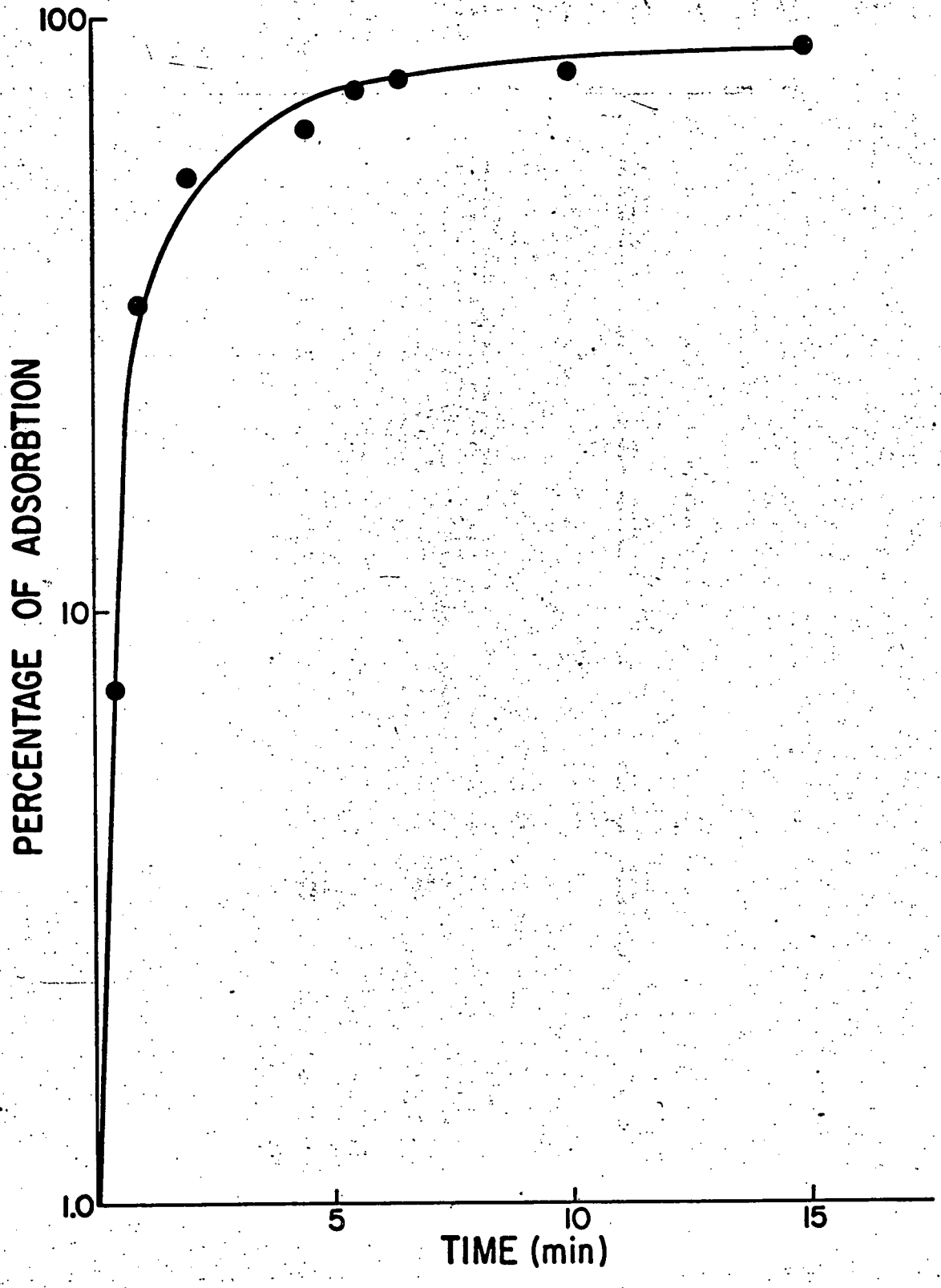

100
10
1.0
0.1
0.01
0.001
PERCENTAGE OF SURVIVAL
10 20 30 40 50 60 70 80
DOSE (seconds)

Figure 6. Adsorption Kinetics of Bacteriophage T7 to E. coli B/r (CSH).


100
10
1.0
PERCENTAGE OF ADSORBTION
5
10
15
TIME (min)

complete, burst abortion was observed as no decrease in turbidity. It was necessary, therefore, to determine at what time post infection to begin the chloramphenicol treatment. The most suitable time would be the earliest time at which most of the phage DNA has been injected. Since it is known that injection of phage DNA commences immediately upon adsorption (Hershey & Chase, 1952), then this can be determined by following adsorption kinetics (Figure 6). Eight minutes post-infection was chosen since over 80% of the phage DNA is injected at that time.

Plating Efficiency

The results of plating efficiency experiments are shown in Table I. The efficiency for UV irradiated phages could not be obtained directly since all hosts are not either hcr^+ or hcr^-. Therefore, UV-irradiated phages must be adsorbed to the four hosts in suspension cultures and the efficiency calculated by subtraction of the unadsorbed pfu. Strain B/r (CSH) was used as the indicator strain and arbitrarily chosen as 100% for the purposes of calculation. Marsden et al (1972) have demonstrated that X-irradiation of hosts has no effect on the adsorption of T4 to B_{s-1} and B.

Post-irradiation Host DNA Degradation

The post-irradiation degradation patterns are shown in Figs. 7-10 for each of the four strains used. Each figure represents a total of eighteen separate experiments for the three conditions. The results of degradation in B and B_{s-1} are in good agreement with those of Marsden et al., (1972) for untreated cells.

In the absence of chloramphenicol and phages, the host degradation proceeds as $B_{s-1} > B_{III-10} > B = B/r$. Chloramphenicol effects are observed for B/r, B_{s-1} and B_{III-10}. No significant change is observed for B.

Table I

PLATING EFFICIENCY

Strain	Unirradiated titer (Adsorbed pfu/ml x 10^{-10})	% Eff	Irradiated titer (Adsorbed pfu/ml x 10^{-3})	% Eff
B/r	4.20	100	9.19	100
B	3.76	89.5	9.52	92.7
B_{III-10}	3.37	80.2	9.08	98.8
B_{s-1}	3.39	80.7	8.64	94.0

Figure 7. Post-irradiation degradation of DNA in E. coli B/r (CSH). Percentage of original activity in acid insoluble fraction vs. time post-irradiation.

● = cells irradiated to 40 kR

■ = cells irradiated to 40 kR + chloramphenicol

▲ = cells irradiated to 40 kR + chloramphenicol + phage T7 irradiated to 800 ergs/mm^2

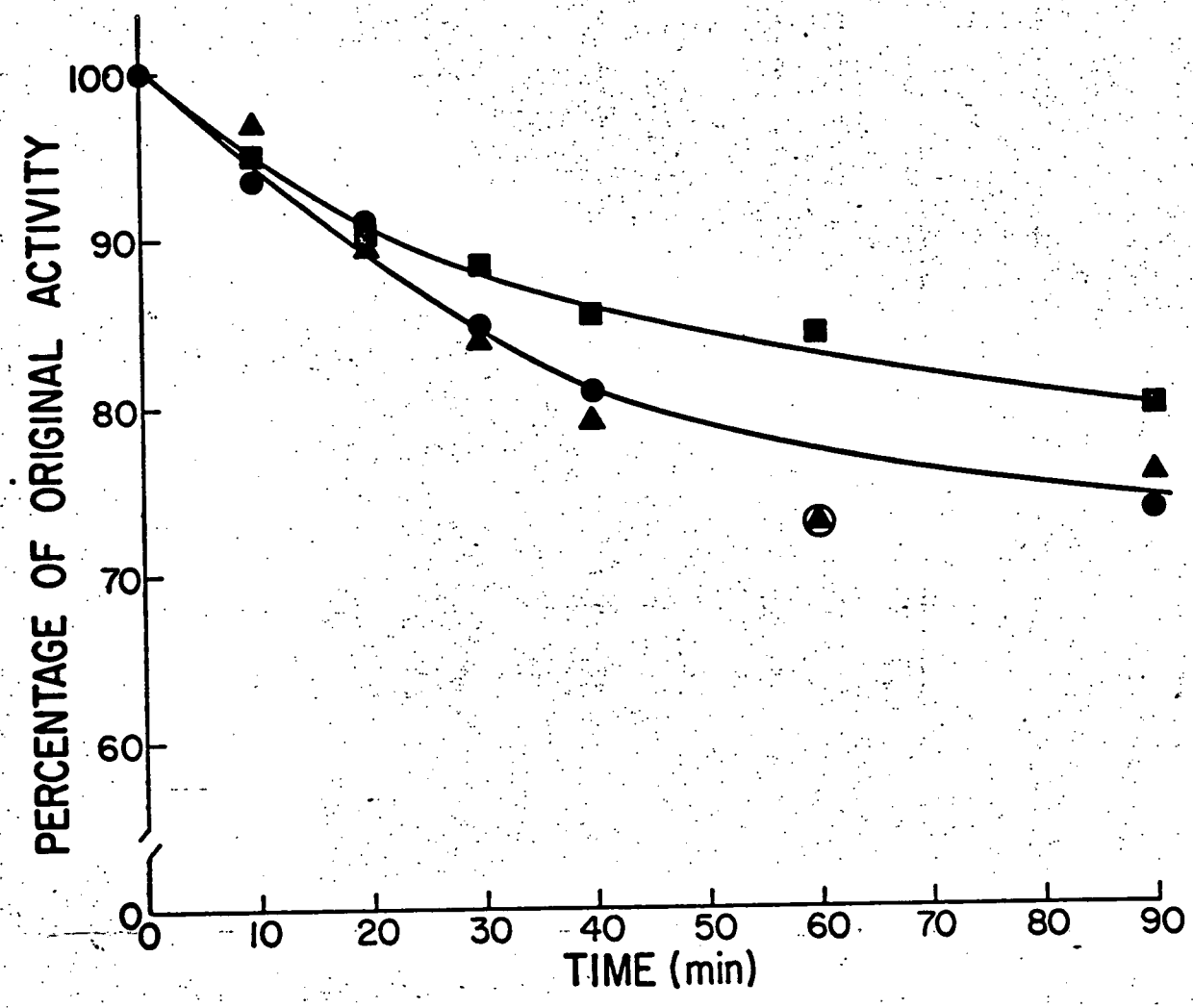

PERCENTAGE OF ORIGINAL ACTIVITY
100
90
80
70
60
0
0
10
20
30
40
50
60
70
80
90
TIME (min)

Figure 8. Post-irradiation degradation of DNA in E. coli B. Percentage of original activity in acid insoluble fraction vs. time post-irradiation.

● = cells irradiated to 40 kR

■ = cells irradiated to 40 kR + chloramphenicol

▲ = cells irradiated to 40 kR + chloramphenicol + phage T7 irradiated to 800 ergs/mm^2

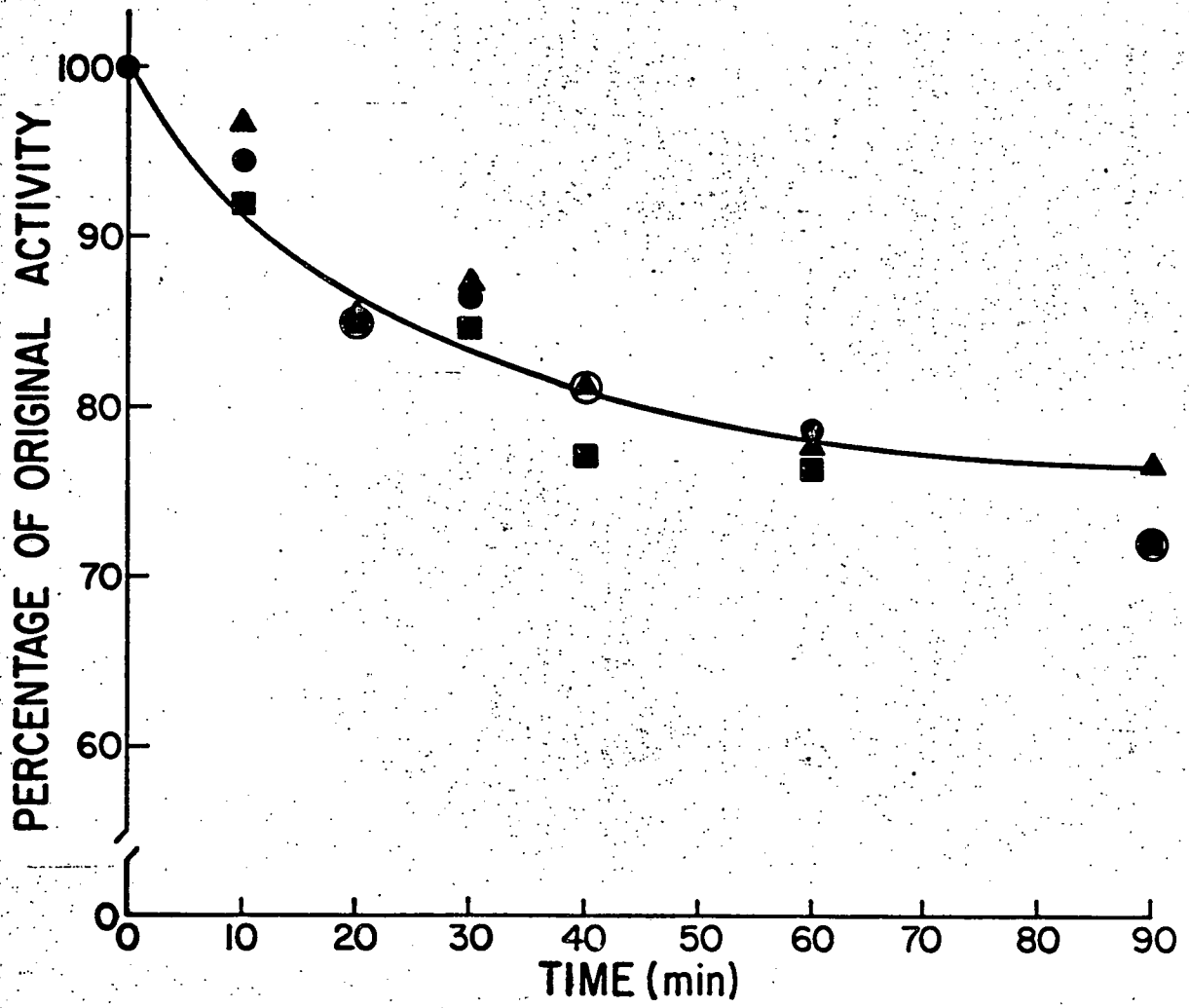

PERCENTAGE OF ORIGINAL ACTIVITY
100
90
80
70
60
0
0
10
20
30
40
50
60
70
80
90
TIME (min)

Figure 9. Post-irradiation degradation of DNA in E. coli B_{III-10}. Percentage of original activity in acid insoluble fraction vs. time post-irradiation.

● = cells irradiated to 40 kR

■ = cells irradiated to 40 kR + chloramphenicol

▲ = cells irradiated to 40 kR + chloramphenicol + phage T7 irradiated to 800 ergs/mm^2

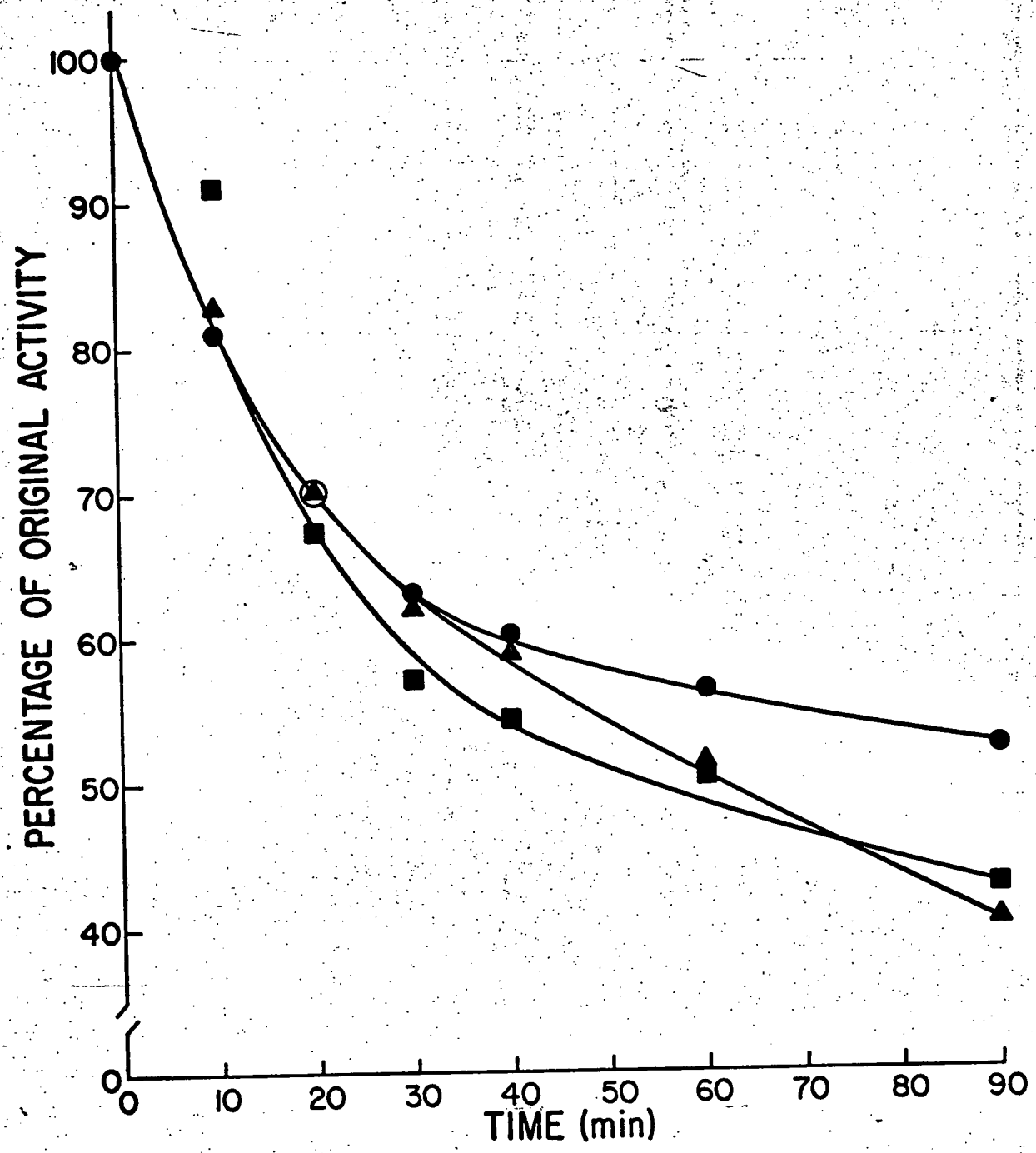
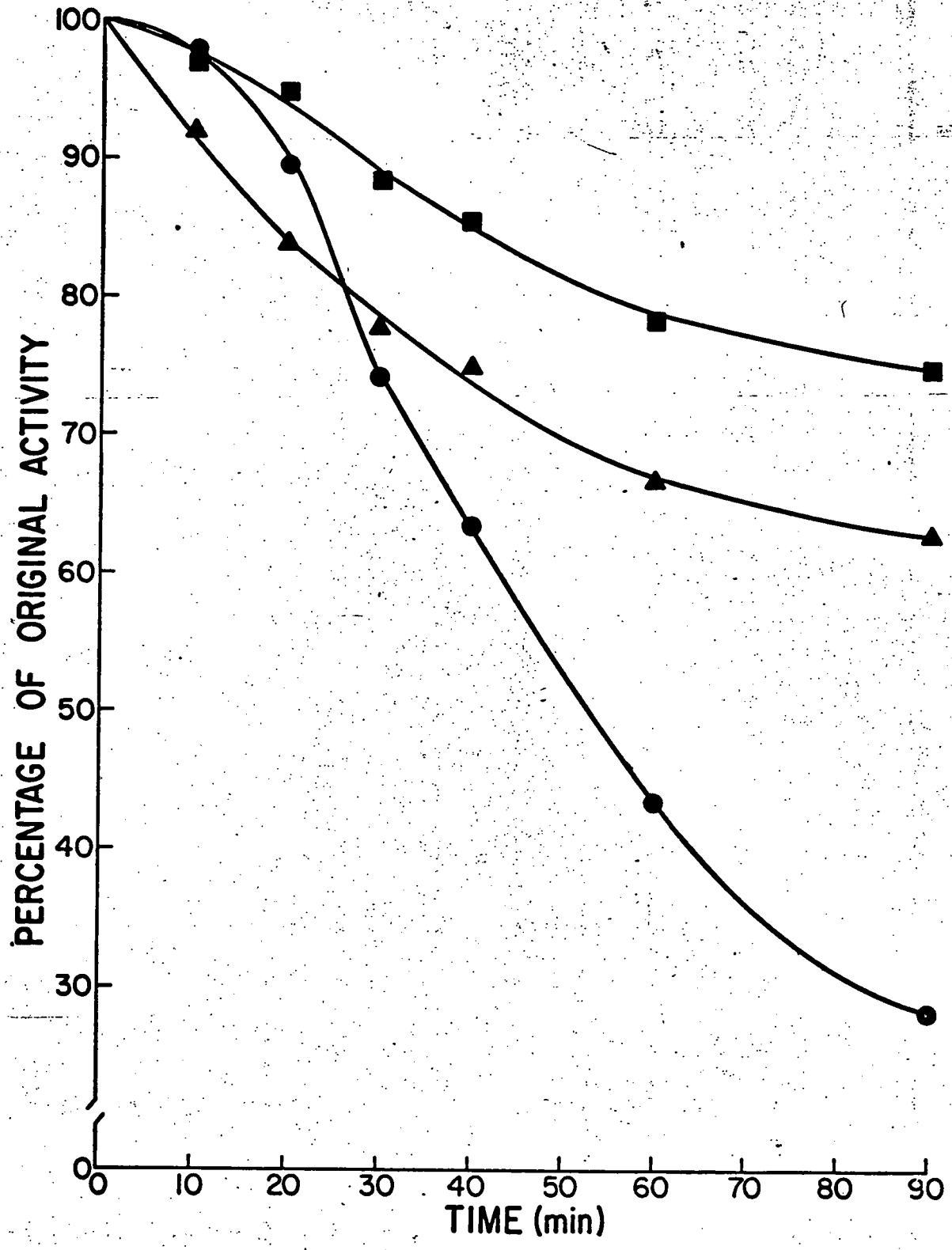

PERCENTAGE OF ORIGINAL ACTIVITY
100
90
80
70
60
50
40
0
0
10
20
30
40
50
60
70
80
90
TIME (min)

Figure 10. Post-irradiation degradation of DNA in E. coli B_{S-1}. Percentage of original activity in acid insoluble fraction vs. time post-irradiation.

● = cells irradiated to 40 kR

■ = cells irradiated to 40 kR + chloramphenicol

▲ = cells irradiated to 40 kR + chloramphenicol + phage T7 irradiated to 800 ergs/mm^2


PERCENTAGE OF ORIGINAL ACTIVITY
100
90
80
70
60
50
40
30
0
0
10
20
30
40
50
60
70
80
90
TIME (min)

Furthermore, the absolute amount of DNA degradation in the presence of chloramphenicol is roughly equivalent for B/r, B and B_{s-1}, ranging from 17.4 to 22.9% of the host genome at 60 min. post irradiation.

The addition of phages results in enhanced degradation with respect to the chloramphenicol treated situation for B_{s-1} and B/r but not for B and B_{III-10}. No direct relations are obvious in comparison of the behavior of the four strains relative to each other.

Dimer Excision

Following an exposure of 800 ergs/mm^2 the percentage of radioactivity recovered from T7 alone as dimers was 1.55%. Since the ion exchange column fractionation method does not allow for separation of types of dimers from each other, but only from monomers (see Fig. 11), the data reflect the presence of thymine-thymine, thymine-cytosine and thymine-uracil dimers.

The results of excision experiments are shown in Table II. Figures in parentheses represent raw data. Figures not in parentheses have been normalized to reflect differences in plating efficiency (Table I) by the following rationale:

> The raw data representa a weighted average of the activity due to unadsorbed phages in the incubation mixture as well as phages adsorbed to hosts. Therefore, P = X (fraction adsorbed) + 1.55 (fraction unadsorbed)
>
> where P = raw percentage recovered as dimer and
>
> X = percentage due to adsorbed phages only
>
> then $$X = \frac{P - 1.55 \text{ (fraction unadsorbed)}}{\text{(fraction adsorbed)}}$$

Dimer excision occurs in all strains. A general trend may be seen relating excision of dimers to survival. That is, the greater the resistance,

Figure 11. Typical elution profile for column chromatography of hydrolyzed DNA. The dimer peak is shown at the left, monomers to the right.

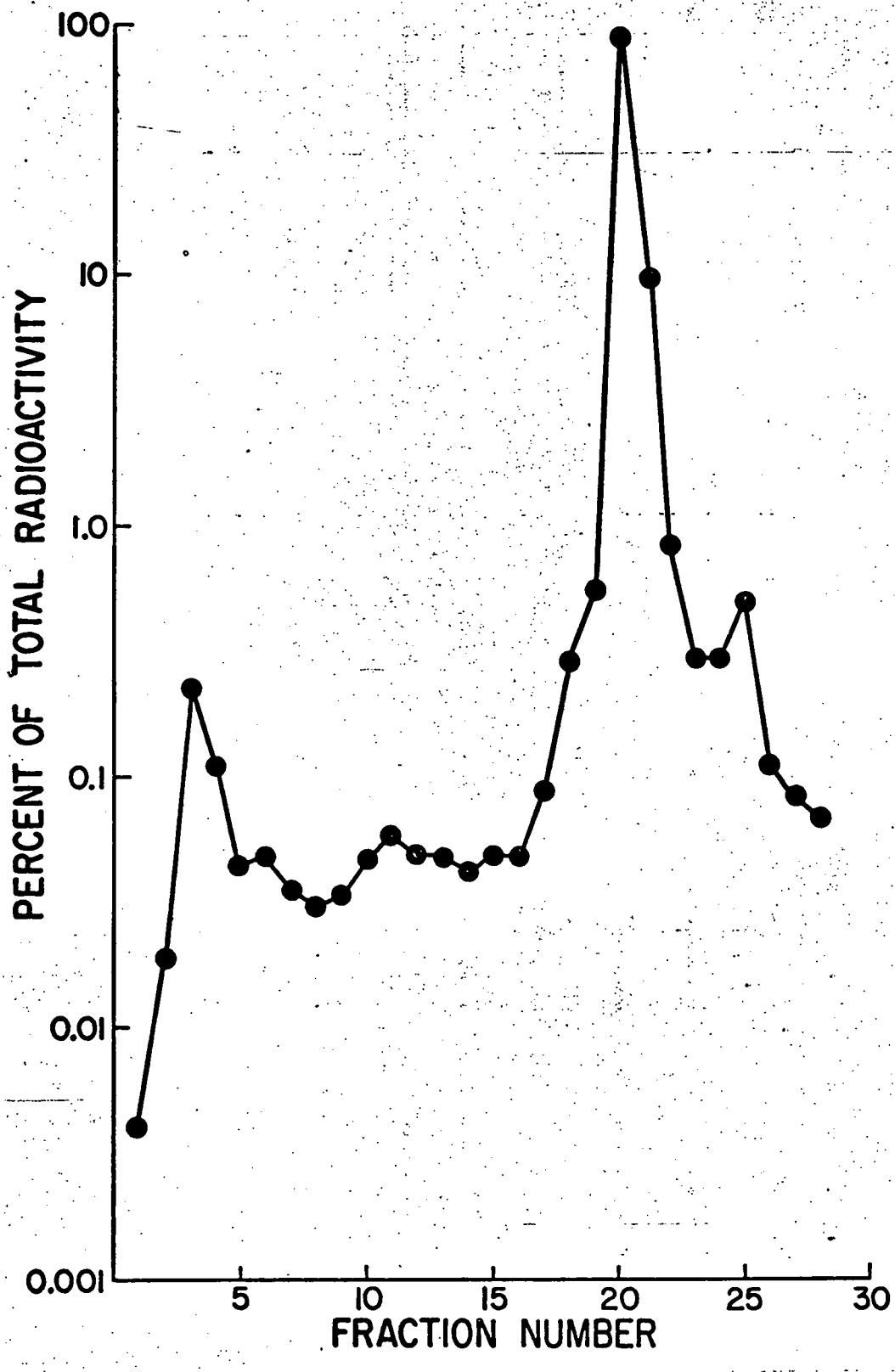

100
10
1.0
0.1
0.01
0.001
PERCENT OF TOTAL RADIOACTIVITY
5
10
15
20
25
30
FRACTION NUMBER

Table II

Percentage of Radioactivity Recovered as Pyrimidine Dimers as a Function of X-ray Exposure to Hosts

Dose / Host	0 kR	10 kR	20 kR	40 kR	80 kR
B/r	(.127), .127	(.109), .109	(.144), .144	(.097), .097	(.138), .138
B	(.241), .121	(.291), .175	(.388), .281	(.386)., 278	------
B_{III-10}	(.603), .592	(.518), .506	(.247), .231	(.392), .378	------
B_{s-1}	(.638), .579	(.414), .341	(.406), .322	(.573), .510	------

Figures in parentheses represent raw data
Measurements taken one hour post irradiation
Normalized data reflect differences in plating efficiency
For explanation see text

the greater number of dimers removed from the phage DNA. This is especially evident at high doses. Significant changes are not observed relating number of dimers removed as a function of dose for each host.

DISCUSSION

Survival Curves

Both UV and X-ray survival curves indicate that the order of resistance is $B/r \rangle B \rangle B_{III-10} \rangle B_{s-1}$, thus confirming the observation by Mattern *et al*, (1966) that the contributions of the exr, hcr, and fil markers are additive. The small differences between B_{s-1} and B_{III-10} for UR survival are in agreement with Witkin (1967 b).

The relative contributions of the three loci may be expressed for X-ray (Fig. 2) curves as a "gene DMF" due to the linearity of the curves. The "DMF" or Dose Modification Factor is usually used to describe the effect of agents added to a population to be irradiated and is used as a convenient expression of the nature of the agent. Agents whose DMF's are greater than 1.0 are said to be protective agents. The presence of a particular locus and therefore a gene product in one strain and not another may be treated in a manner to other defined agents. That is, the 10% survival value of a strain possessing the locus divided by the 10% survival value of a strain isogenic except for that locus would give the "gene DMF". Thus $DMF_{fil} = \frac{LD_{90}\ B/r}{LD_{90}\ B}$, $DMF_{hcr} = \frac{LD_{90}\ B}{LD_{90}\ B_{III-10}}$, and $DMF_{exr} = \frac{LD_{90}\ B_{III-10}}{LD_{90}\ B_{s-1}}$. Under these conditions, the DMF's are 4.5, 12. respectively. Goldstein *et al*, (1973) have determined these gene DMF's to be 2.5, 1.04 and 1.7 for stationary cells irradiated in buffer. It may be seen that the relative effect of the Fil^- phenotype again is vastly greater than those of the other two. The relation of this to the unique recovery processes associated with the Fil^- phenotype will be discussed later.

Since the UV survival curves are not strictly linear on a semilog plot,

calculation of DMF is not applicable. It should be noted, however, that the curves for B_{s-1}, B_{III-10} and B break in the same direction while that for B/r has a shoulder. The data are in general agreement with those of Witkin (1967 b). The largest contribution to survival is made by the presence of the Hcr^+ phenotype (Figs. 3 and 4). That the hcr function plays a greater part in UV survival than X-ray survival is to be expected since the hcr gene product is probably an endonuclease (Friedberg, 1972; Ellison et al, 1960). That B_{III-10} and B_{s-1} have similar UV survival curves led Witkin (1967 b) to postulate that the exr function is also an endonuclease. These results are confirmed by the present X-ray and UV data, since a great difference between B_{s-1} and B_{III-10} survival curves is not seen for X or UV irradiation.

Similarities have been noted (Witkin, 1967 a) between the processes of filament formation in the B series and prophage induction in the K-12 series. The observation of sectoring following low doses of UV has been described earlier. Prophage induction has been observed following low exposures of K-12 lysogenic for lambda to UV (Jacob and Fuerst, 1958) and this is now a widely used procedure (Stent, 1963). It is not conclusive whether the phenomenon observed is actually UV mediated prophage induction, but the following points strongly implicate that this is the case:

1. Sectoring was observed for all strains carrying the Fil^+ phenotype (B, B_{III-10} and B_{s-1}) but not for Fil^- (B/r).

2. The ratio of sectored area to colony increased with time. This would be expected if prophage inductions occurred since the phage generation time is shorter than the host generation time.

3. Sectoring was observed only following low doses of UV. No sectoring was seen at higher doses. Similar observations have been made regarding

prophage induction by Lwoff et al., (1950).

4. The colony morphology of unirradiated cells infected with T7 and immediately spread on plates was strikingly similar to the UV-induced sectoring. There are two possible explanations for this: either the induced prophage gives a plaque morphology similar to that of T7, or T7 is acting as a "helper phage". It is not clear at this time which alternative is correct.

Cohen (1959) observed an apparent prophage induction in E. coli B and C. The prophage (as determined by dismunity studies) is a varient of the Shigella phage P2. She was not able to detect, however, any mature or defective progeny phages. Rupert and Harm (1966) as well as Witkin (1967 a) have suggested the possibility of the involvement of defective prophages as one of the reasons explaining the differences in survival of B/r and B. Grady and Pollard (1968) were able to cure E. coli 15T$^-$ of its defective prophage. Their findings that the cured derivitives showed a decreased post-X-ray DNA degradation led them to postulate that the cessation of degradation associated with prophage induction serves to increase the probability of cell survival. This hypothesis required, then, that B and B/r harbor a defective prophage while B_{s-1} does not. Furthermore, they quote Cohen's study (1959) and imply that her strain C and B/r are one and the same. This is clearly not the case since the B/r used here is not a mating strain whereas C is. Many radioresistant derivatives of B isolated independently have been denoted B/r (Adler, 1966) also making it unlikely that the strains are the same.

The Witkin hypothesis relating ability to induce prophages to the Fil$^+$ phenotype does not, however, require that Fil$^-$ strains be devoid of

defective prophages, but only that they be non-inducible. That sectoring was not observed for our B/r strain does not mean that prophage induction is absent under all circumstances, although it would be convenient to think so--especially in view of the DNA degradation picture for strains B/r and B (Figs. 7 and 8). That is, for untreated cells, the time courses of percent original activity remaining in high molecular weight DNA coincide for B and B/r.

Post-Irradiation DNA Degradation

Untreated Cells - For the sake of clarity, the curves of post-irradiation degradation vs. time for untreated cells of all four strains are redrawn from Figs. 7-10 and presented in Fig. 12. It may be seen that the general rule relating X-ray sensitivity to post-irradiation breakdown of DNA seems to hold for the hcr^+ and exr^+ loci when degradation is measured at times greater than 40 minutes post-irradiation. Furthermore, at the end of the time course, where the curves begin to level off, it is found that B_{s-1} (lacking both hcr^+ and exr^+) retains 27% of its genome, B_{III-10} (lacking exr^+) retains 53% of its genome and B (lacking neither) retains 73.5% of its genome. It appears, then, that the contributons of those two loci toward post-irradiation breakdown are additive and practically equal. The implication, then, is that the gene products, which are both presumed to be endonucleases, do not compete for the same sites on the DNA. It is interesting to note that the gene DMF's are also practically equal (1.2 for hcr and 1.1 for exr). These correlations, therefore, support the idea that DNA breakdown is, in fact, related to survival.

In contrast to this is the fil^- contribution. The degradation curves (Fig. 12) of B/r (fil^-) and B (fil^+) coincide and it would seem

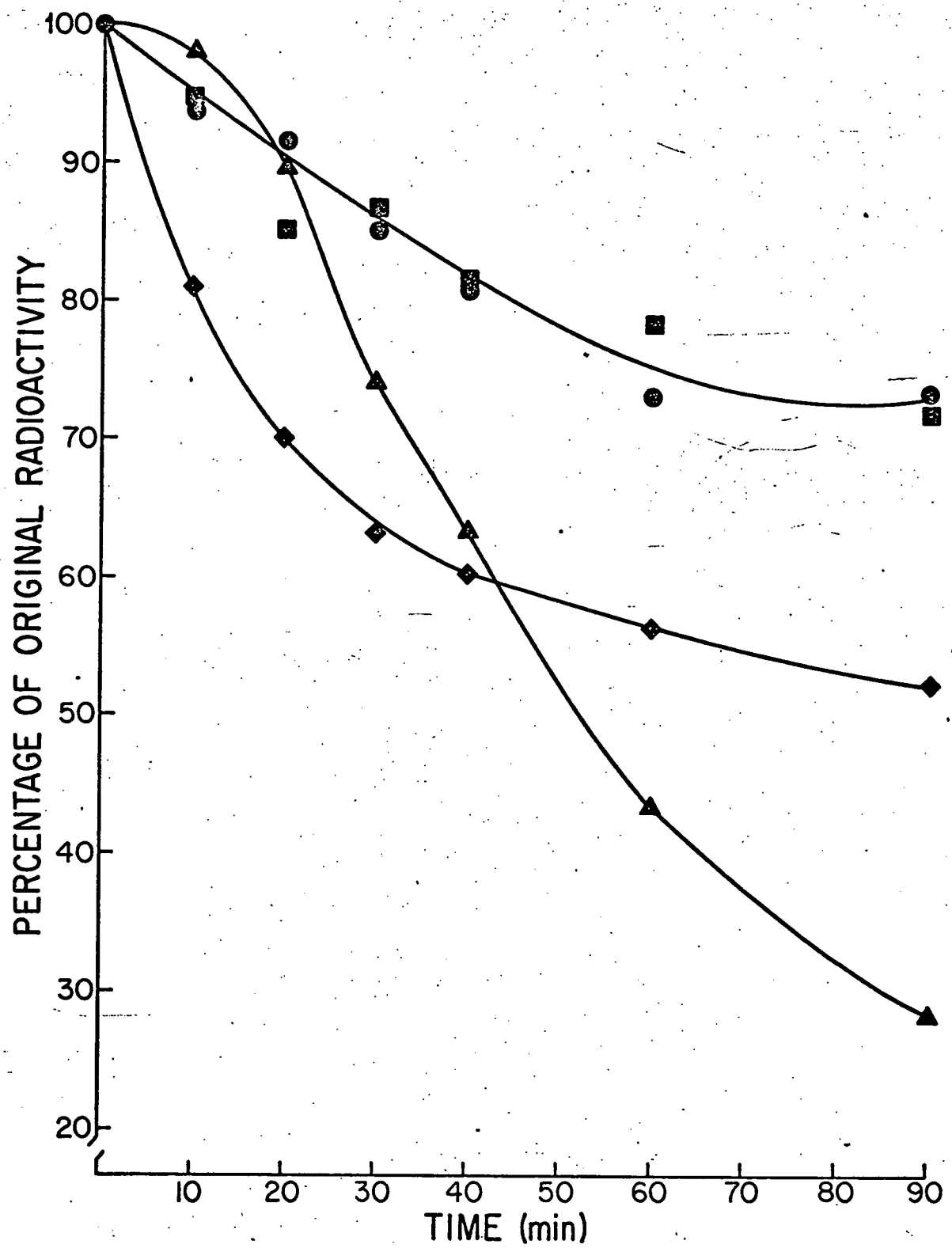

100
90
80
70
60
50
40
30
20
PERCENTAGE OF ORIGINAL RADIOACTIVITY
10
20
30
40
50
60
70
80
90
TIME (min)

Figure 12. Post-irradiation degradation of DNA in untreated E. coli. Percentage of original activity in acid insoluble fraction vs. time post-irradiation.

● = B/r (CSH)

■ = B

◆ = B_{III-10}

▲ = B_{s-1}

that the Fil⁻ phenotype effect is related to something other than breakdown. Investigations in other laboratories further confuse the issue. Recently Horan et al, (1972) have isolated a fil⁻ mutant of B_{s-1} which exhibits almost no post-irradiation breakdown after doses of 20 kR or less, and only minimal degradation after doses above 20 kR. The mutant (PHHI) is more radioresistant than B/r but has a peculiar breakdown pattern at high doses (60-200 kR). Even at exposures above 20 kR but below 100 kR no absolute relationship is apparent between survival and breakdown, and certainly, no correlation exists between extent of breakdown and survival levels in relation to other strains of the B series.

Chloramphenicol Effects

Blockage of protein synthesis post-irradiation has a decided effect on DNA breakdown patterns although the nature and extent of the effect depend on method of treatment and experimental conditions. Early reports on the effects of chloramphenicol treatment (10 μg/ml) in stationary E. coli B (Miletic et al, 1961; 1964) indicate an enhancement of degradation with time. If the rate of protein synthesis is depressed by growth at low temperatures, DNA breakdown is also depressed, but if the rate is depressed by growth in succinate medium rather than glucose medium DNA breakdown is enhanced (Achey & Pollard, 1967) in E. coli 15T⁻. Other studies with log phase 15T⁻ (Frampton & Billen, 1966) lead to confusing results. In supplemented medium, the expected degradation pattern was observed over a 5-40 kR range. That is, the extent of degradation after 60 min. is directly related to dose. If amino acid starvation is used to arrest protein synthesis, the extent of degradation is increased for each dose studied. The linearity observed for cells in

supplemented medium, however, does not hold. The final degradation at 60 min. is greater for cells receiving 10 kR than 40 kR; greater for 20 kR than 30 kR; greater for 20 than 40 kR. These experiments were repeated using populations with chromosomes "aligned" before irradiation by the amino acid starvation method. Of these, degradation followed the same order with respect to dose as the nonaligned, amino acid starved populations above. The extent to which DNA was degraded for each dose was drastically reduced. Furthermore, when aligned chromosomes are used, if protein synthesis post-irradiation is blocked by amino acid starvation, DNA breakdown is greatly enhanced relative to cells in fully supplemented medium post-irradiation. Similar observations were made in K-12 with regard to amino acid starvation and decreased breakdown (Emmerson & Howard-Flanders, 1965).

Due to the conflicting published data relating altered protein synthesis and post-irradiation breakdown, it was necessary to study this phenomenon under the present experimental conditions. Chloramphenicol treatment has the most marked effect in B_{s-1} (Fig. 10) where breakdown is reduced from 72% to 25% of the genome. Breakdown is also reduced (27-20%) in B/r, enhanced (47-57%) for B_{III-10} and unaffected in B after 90 minutes incubation post-irradiation. The observation of Miletic et al., (1961) that chloramphenicol resistance may be measured as an increase in turbidity of treated cultures with time, was used to ensure that all strains were, in fact, sensitive. The results (data not shown) indicate that they were. The apparent gene DMF—percent breakdown correlation seen for untreated cells is not evident.

At present, no suitable explanation exists with respect to the relative action of chloramphenicol on these four strains. The opposite chloramphenicol

effect in B_{s-1} and B_{III-10} (Figs. 9 and 10) does, however, support the above idea of non competition of the exr and hcr gene products. Moreover, the enhancement effect in B_{III-10} and the depressed effect in B_{s-1} are not equal and opposite, i.e. they do not sum to the negligible effect in B implying that B does not have a double dosage of the same gene product. That B/r shows a depressed breakdown but B does not (Figs. 7 and 8), further indicates the dissimilarity of the exr and hcr functions. These data also support the likelihood that the fil^- function is distinctly separate from those of exr and hcr.

Phage Competition

The ultimate success of this type of experimental approach obviously depends on the attachment of host enzymes to phage DNA. It is likely, therefore, that this would be reflected as a competition owing to the commonality of post-UV and post-X ray repair processes. That is, assuming that nucleases are saturated with substrate, the introduction of unlabeled phage DNA should result in a depressed extent of host degradation with respect to the chloramphenicol treated situation. Clearly (Figs. 7-10) this is not the case.

It is not likely that the observed enhanced degradation is related to a survival effect (different percentages of survival in the four strains at the 40 kR exposure). Frampton and Billen (1966) have determined in 15 T^-, at least, that the initial rate of degradation is constant for cells irradiated from 10-40 kR. Nor is it likely that enhanced degradation is due to participation of phage-directed enzymes. Studies with B and B_{III-10} (Figs. 8 and 9) indicate that where there is no chloramphenicol effect, there is no phage effect either (B) and where there is a reverse chloramphenicol effect (B_{III-10}) the phage effect is also negligable.

The final extent to which the DNA is degraded depends on either the level of nucleases or the level of "stop degradation" control function or both. On a per cell basis, DNA degradation is apparently an "all-or nothing" type phenomenon (Hildebrand & Pollard, 1969; Pollard & Tilberg, 1972) in at least two strains of E. coli (15 T$^-$ and B_{s-11}). That is, the degradation patterns observed here are dependent upon the relative percentages of the population which do or do not degrade. In view of this evidence, then, it would seem that the presence of chloramphenicol serves to shift these percentages in three of the four strains. This being the case, that the populations of untreated and chloramphenicol treated cells are not identical, the addition of phage to the system must be analyzed only with respect to chloramphenicol treated populations.

If the hypothesis of Kelly et al, (1969) is correct, then the "stop degradation" control is the ability of a ligase to displace the nuclease/polymerase from the DNA. Phage effects are apparent in B/r and B_{s-1} and result in enhanced degradation. The implications of this are twofold. First, since the competition leads to enhanced rather than depressed degradation, the phage DNA must compete for ligase to a far greater extent than nuclease. Secondly, since the competition is considerably more pronounced in B_{s-1} as opposed to B/r, the ligase activity must be greater in B/r than B_{s-1}. This idea is supported by the strand break restitution experiments of McGrath and Williams (1966).

The absence of phage effects in B and B_{III-10} might then be interpreted to mean that elevated ligase levels are present in those two strains. Whether or not this is correct is not clear. Alkaline sucrose experiments have never been performed for B_{III-10}. Such comparisons, however, have been made between B and B/r (Goldstein et al, 1973). Their findings indicate

that differences do not exist in ultimate strand break restitution for populations not treated with chloramphenicol. That the degradation profiles for B and B/r in the presence of phage are identical (Figs. 7 and 8) and also identical to the untreated situation suggests that ligase activity in B is at a supersaturating level.

Goldstein et al., (1973) have isolated a radioprotective extract from M. radiodurans which was tested on this four strain series of E. coli B derivatives. They found that the extract exerted the greatest protective effect on the fil⁻ "gene DMF" but no effect was observed on the alkaline sucrose profiles either in relation to the untreated controls (B and B/r) or to each other. These findings, then, offer strong support for the idea that the Fil⁻ contribution operates at some level other than the DNA degradation.

Dimer Excision

The number of dimers produced by UV-irradiation is obviously dependent on the actual dosage to the sensitive target. The in situ condition of the sensitive target, then, is the determining factor. It would be expected that the greater the amount of "shielding material" protecting the DNA (i.e. cell size, protein mass etc.) the smaller the number of dimers produced by a given exposure. Howell (1972) found that 50,000 ergs/mm^2 was necessary to produce 3% dimers in whole cell preparations of M. radiodurans. In contrast, 11.6% of the activity was present as thymine containing dimers after exposure of naked transforming DNA to 15,000 ergs/mm^2 (Bron and Venema, 1972 c). The present value, then, of 1.55% is in reasonable agreement for an exposure of intact phages to 800 erg/mm^2.

That repair takes place in all four strains studied is demonstrated by the fact that none of the values in Table II approximates 1.55%. Of special interest is the observation of repair in B_{s-1}. Whole cell studies of repair in B_{s-1} have been difficult due to the massive host degradation (Fig. 10). The cell's enzymes, however, are capable of dimer removal from phage DNA, implying that any repair taking place in the host is masked by degradation. In support of this idea is the observation of strand-break restitution in PHHI (Horan et al, 1972) by the alkaline sucrose method.

The capacity of γ-irradiated hosts to support phage growth has recently been studied by Marsden et al, (1972). Although on a populational level the capacity to support phage growth seems to fall off with time and parallel host DNA breakdown (in B_{s-1} but not B), on a per cell basis, this is an all-or-nothing phenomenon. Neither ability of irradiated hosts to adsorb T4 nor injection of phage DNA are interrupted. Rather, the sensitive processes are transcription and translation of the phage genome. Their assay was for the ultimate appearance of phage-directed dCMP hydroxymethylase and, therefore, it was not possible to distinguish between the two processes. Earlier work (Boyle & Setlow, 1970; Swenson and Setlow, 1966) indicates that chloramphenicol has no effect on dimer excision from cellular or phage DNA. It is attractive to assume, at least in the case of the present studies, that non-DNA related processes do not enter into the picture.

Table II indicates that while repair ability is not destroyed as a function of dose, the extent to which dimers are excised increases with increasing radio-resistance as measured by survival. No generalizations other than this, however, are apparent. The relations to gene

DMF and post-irradiation host degradation are not evident. It is noteworthy that dimers are excised to a greater extent in B/r than B while the host breakdown patterns are coincident. This may be explained by the observation of Boyle and Setlow (1970) using a K-12 lambda system. They found when UV irradiated hosts were infected with UV-irradiated lambda, dimers in lambda limit the excision of bacterial dimers better than the converse situation. They interpret this to mean that there is an unequal distribution of repair enzymes within the cell. Such an explanation is not inconsistent with the idea that the contribution of the Fil$^-$ phenotype is not directly concerned with DNA effects.

If repair ability is not destroyed by exposure to ionizing radiation what then is the relation of repair to the ultimate lethality or survival of the irradiated host? Survival levels cannot be completely correlated to post-irradiation breakdown. If the hypothesis of Kelly et al (1969) is correct (and it is substantiated by the present work), then depolymerization/repolymerization is controlled by the same enzyme system and should, therefore, be stoichiometric. Previous experiments attempting to relate post-X-ray depolymerization to repolymerization have not revealed this stoichiometry. Such data, however, must be reinterpreted in light of Billen's (1969) finding that semiconservative duplication is initiated post-X-ray. This being the case, repair replication by itself as observed on a populational level would not be precisely related to survival levels. The general trend seen in Table II, that depressed survival levels are concommitant with depressed "repair ability", argues strongly that the ultimate lethality is determined by an inability of the cell to cope with the amount of damage presented, rather than destruction of the ability to repair the damage.

SUMMARY AND CONCLUSIONS

Radioresistance is controlled by at least three genes in the B series of E. coli. In order to determine whether the repair system is damaged by exposure to ionizing radiation, X-irradiated hosts were infected with UV-irradiated phage T7. The ability of hosts to excise dimers was measured as a function of dose. Work done involves a comparison of hosts differing in the three loci.

1. X-ray survival of the four strains E. coli B/r (CSH), B, B_{III-10} and B_{s-1} in log phase follows the same relative patterns as stationary phase (Goldstein et al., 1973).
2. The contributions of the three markers, fil, exr and hcr to survival are additive. The gene products of the exr and hcr loci probably perform similar functions but act at different sites on the DNA.
3. Prophage induction or a mimetic phenomenon was observed following low doses of UV for the fil^+ strains, but not for fil^-.
4. UV-irradiated phage T7 competes for some repair function which is probably not a nuclease with the bacterial genome of hosts B/r and B_{s-1}, but not with B_{III-10} or B.
5. Ability of hosts to excise pyrimidine dimers from phage T7 is not destroyed by increasing exposure.
6. Percentage of dimers remaining in phage DNA after 60 minutes incubation with hosts is related to the radiosensitivity of the host.

APPENDIX

Media

Nutrient (NUT) Broth
per liter - 8.0 g Nutrient Broth (Difco)

Nutrient (NUT) Agar
per liter - 23.0 g Nutrient Agar (Difco)

Nutrient Top Agar
per liter - 8.0 g Nutrient Broth
8.0 g Agar (Difco)

M-9+
per liter
2.5 g hydrolyzed casein (Sheffield)
13.0 g Na_2HPO_4
3.0 g KH_2PO_4
1.0 g NH_4Cl
250 mg deoxyadenosine (Calbiochem)
add sterile per liter
1.0 ml 1.0 M $MgSO_4$
2.0 ml 25% NaCl
0.3 ml 0.01 M $FeCl_3$ in 0.1 N HCl
1.0 ml 2% gelatin (Difco)
5.0 ml 20% glucose

Liquid Scintillation Fluor
per liter - 333 ml Triton X-100 (Packard)
667 ml Toluene
5.5 g PPO (Baker)
0.1 g POPOP (Baker)

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