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RADIATION DAMAGE AND REPAIR IN CELLS AND CELL COMPONENTS

Progress Report

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and

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First new contract year

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Progress Report: First New-Contract Year

INTRODUCTION

Our collaboration is that of two investigators with considerable experience in effects of both ionizing radiation and ultraviolet light on both living systems and components of living systems. Our approach, that of many other investigators, is much aware that radiation does not simply damage the cell in an all-or-nothing way; the cell has remarkable facilities for repair of damage produced both by ultraviolet light and by ionizing radiation. It is becoming apparent that chemical agents can also elicit such repair processes and that their action has to be interpreted with an appreciation that the cell can remove the damage which they produce. Indeed, the degree to which repair processes are active on the DNA of the cell has come as a considerable surprise, a shock even, to those who first recognized DNA as the hereditary material of the cell.

Understanding the response of cells to DNA damage will take many laboratories many years of study. Such studies may bring understanding of agents which cause transformation of cells and hence of cancer. With this end in view we are putting particular emphasis on the study of one aspect of repair, in part discovered by one of us, and with which we both are well prepared to work. Induced repair, also called error-prone repair and sometimes SOS repair, is characterized in Appendix Ia, an overview of all of these phenomena which will be part of a monograph on the subject of induced repair, to be published by Marcel Dekker. A useful summary is thus

provided to indicate the areas of work which are of importance to us.

By way of further indicating our particular involvement with induced repair, we include a page or two of summary (Abb. Ib) prepared for the Department of Energy at their recent request. We recognize five areas important to us and to which we relate our work. But first, why are we putting all of the effort of our laboratory into this general area? The reason is that our interpretation of past experience with radiation phenomena, and other pheonmena, suggests a subtlety in the response to radiation which goes well beyond the original phenomenon. It is not sufficient to say simply that the cell contains repair processes and that these may take time. Such explanations do not easily cover the phenomena of double exposure experiments and they do not readily relate to the fact that a phenomenon like cancer can develop after many years following the original dose of radiation. We sense in the recent discovery of a repair process which is not constitutive in the cell, but one present as an alteration produced by the radiation itself, an exciting new potential. This new aspect of radiation effects can help explain the rather obscure and strange behavior of cells toward radiation, including those other factors mentioned above.

It may be that this phenomenon, observed principally in bacteria, is not general and that it will not play a part in the understanding of radiation effects on eucaryotic cells and on plants and animals. However, we find a potential for explanation of the strange effects of radiation exceeding any other up to the present. We feel that it is worth our time and interest to spend some years on the study of this phenomenon.

Specific accomplishments of the past contract year can be summarized under seven headings.

SPECIFIC ACCOMPLISHMENTS

I. Coordinate Induction

Our work on the coordinate character of induced repair has been presented most recently at the Keystone Conference. The question we ask is whether all induced phenomena go together when induced by a single inducing agent. Our previous explorations of this question looked at induced mutagenesis, induced radioresistance, and induced inhibition of post-radiation DNA degradation to see whether these three were induced together. We concluded that they reasonably did occur in coordination. We had some indication that the earliest phenomenon observed, the induction of phage lambda, follows an induction kinetics distinct from that of the other processes. Subsequently, we turned to a strain in which the lambda lysogen can readily be produced and studied four phenomena in closer comparison in the same cell strain: W-reactivation, the induction of phage lambda, the induced inhibition of post-radiation DNA degradation, and induced radioresistance. We found that three of these phenomena do coordinate, but that the induction of lambda phage is apparently substantially more sigmoidal in character. Lysogenic induction, if coordinate with the others, has some additional complicating steps or constraints. This work is to appear in the proceedings of the Keystone Conference; and is included as Appendix II.

II. Filamentation.

Through the interest of an excellent Duke University undergraduate

(Brian Robinson) in this work, it has been possible to add filamentous growth to the list of inducible processes we are studying. The study of filamentation, added to the above mentioned four manifestations of induced repair, can more conclusively test coordinate behavior. Microscopic observations of cells in a Petroff-Hausser counting chamber, with microphotographs for analysis, has been put into good working shape by Brian Robinson. The procedure used is to give a small inducing dose of ultraviolet light and then to follow the appearance of the cells under the microscope. Pictures taken at intervals are subsequently analyzed for the length of the cells. A normal culture of E. coli which has not received ultraviolet light has cells varying in length by about a factor of two but characteristically quite short. Cells in which something has happened to septum formation behave differently. If the cells are not mutant in the property of forming filaments the increase in length does not usually exceed four times that of the normal cells. Cells mutant at the lon locus have the property of forming long filaments ("snakes"), readily scored as true filaments. The induced effect on septum formation has been studied thus far in four cell genotypes: uvr^+lon^+, uvr^-lon^+, uvr^+lon^-, and uvr^-lon^-. Figure 4 in Appendix III shows the effect of giving different doses of ultraviolet light on observing the fraction of cells yielding some effect on septum formation in these four strains. The induction kinetics are not all the same. In particular, uvr^-cells are more readily induced at much lower doses but show decline in effect at larger doses. Figure 4 also shows that introduction of the lon mutation in the uvr^+ strain also increases inducibility at lower doses. For

the uvr$^-$ strain this increase has been explained in a greater prevalence of daughter strand gaps where dimers are not excised before DNA replication. It is difficult to maintain this explanation for the uvr$^+$lon$^-$ case, particularly since Adler has shown that a diffusable component from lon$^+$ cells can supply what is missing and cause the septum to form.

An interesting potential in this work is the study of cell size distribution. Since the cells are photographed it is possible to make a detailed analysis of the distribution of sizes and also to consider whether induction of filamentation is an all-or-nothing process. This analysis is still quite preliminary but it can be said that there is quite reasonable suggestion of the all-or-nothing interpretation. At relatively small doses there is a fraction clearly not filamenting as well as a fraction obviously filamenting very well. A further remarkable indication is that size distribution is not a continuum; specific lengths are indicated. Some degree of synchronization of cell elongation is suggested, consistent with previous work not yet published.

Appendix III represents Robinson's interim report in a study which will continue.

III. Action Spectrum for induction of K12λ.

Since the induction of lambda suggests some differences in kinetics from other inductions a multiple approach was worked out, looking toward study of the wavelength dependence of lysogenic induction. The only available action spectrum for inductionof lambda was done sometime ago by Richard Franklin. Its odd aspects and the further recognition of complications in quantitating lysogenic

induction have prompted its repetition. If the induction of lambda is multi-hit then its interpretation might involve changes in the action spectrum induced. Three indicators of the induction process have been used in this extended study. One is the plating of infectious centers. UV-induced lysogenic cells are plated on an indicator strain (here E. coli Q_1), giving rise to plaques. The relative number of plaques versus the number of viable cells is an indication of the percent of cells which have been induced as infectious centers. A second measure involves cell lysis. If the cells have previously grown on lactose they contain induced levels of the enzyme β-galactosidase, detectable by a yellow color with an indicator substrate ONPG. However, intact cells, even though they contain this enzyme, do not give the color to any large extent. Cell lysis allows enzyme and color reagent to mix and color develops rapidly. The method is quite sensitive. The third indicator of lysogenic induction is the killing of the cells. Cells induced for lambda do not form colonies, a third evidence of induction.

Figs. 1&2 show that the dose responses for induction at a particular wave length for the three phenomena observed do follow a characteristic induction curve. They are not exactly the same and there is some evidence that the physiological state of the cells can influence the separation of the different responses.

Figure 3 shows an action spectrum for seven wavelengths, derived by measuring the ultraviolet dose necessary to give 50% of the maximum induction and plotting the reciprocal of that dose as a measure for inducibility. We include in this figure the data of Franklin for comparison. This action spectrum, still preliminary, shows differences both from the absorption spectrum of DNA and

also from Franklin's work. More work is needed and is intended.

IV. Work on Ultraviolet Light in the Frozen State.

Appendix V , an abstract from the Sixth annual meeting of the American Society for Photobiology, describes work with UV on cells induced in the frozen state.

V. Wavelength Dependence of Mutagenesis.

Appendix VI , another abstract from the same meeting, summarizes our further work on the action spectrum of UV mutagenesis.

VI. W-reactivation of X-rayed Phage Lambda.

Appendix VII represents work on w-reactivation of X-ray-damaged phage, both protected and less protected.

WRITING IN PROGRESS

(a) Chapter on CRC book on Radiation Sterilization. "Action of radiation on cells and tissues". Essentially complete.

(b) Volume II of "Contemporary Biophysics", Dekker. Radiation Induced Repair (E. C. P. and R. C. Bockrath).

(c) Collection of material on wavelength dependence of mutagensis.

(d) Paper with P. M. Achey on Oxygen vs Nitrogen effect of induction of radioresistance.

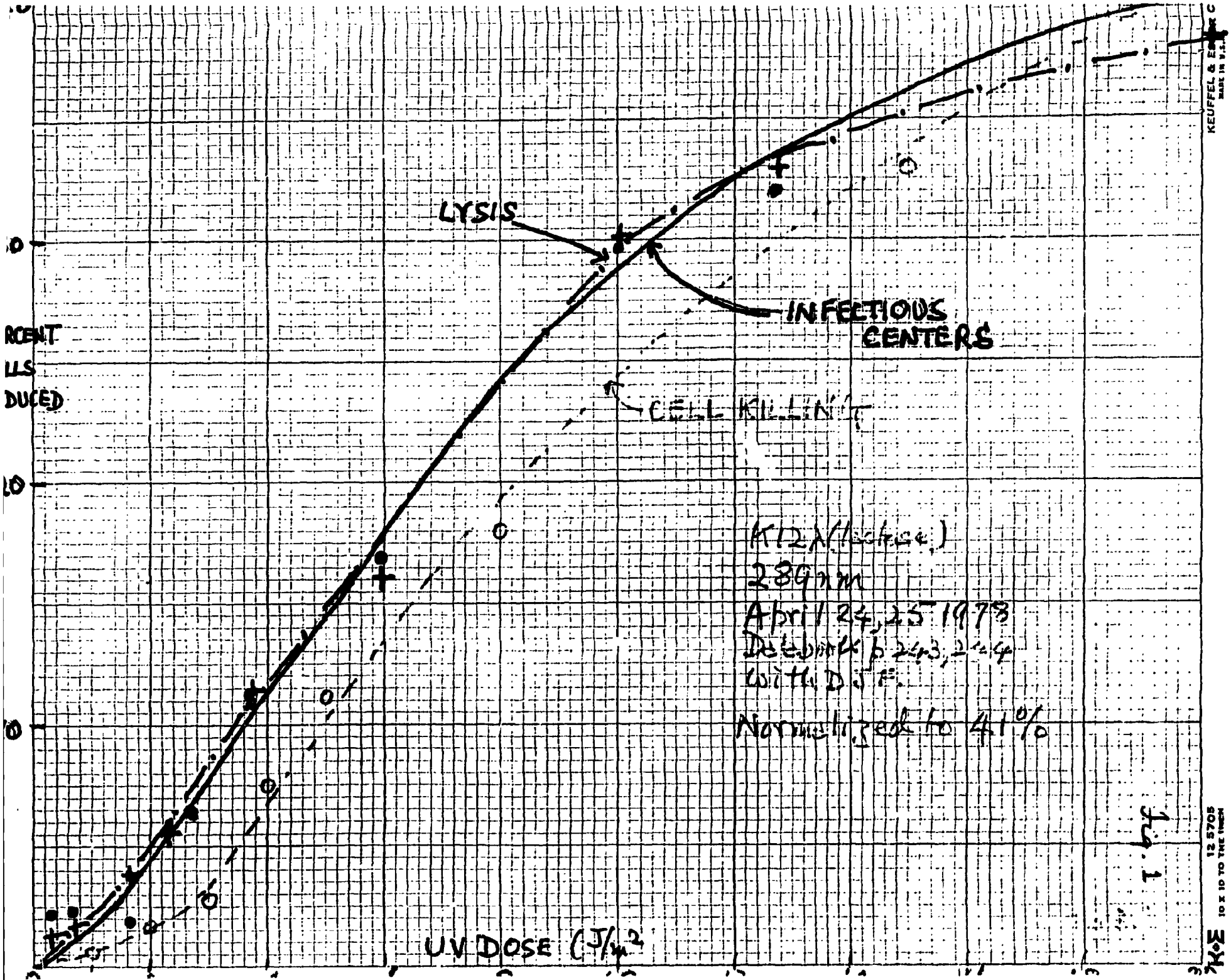
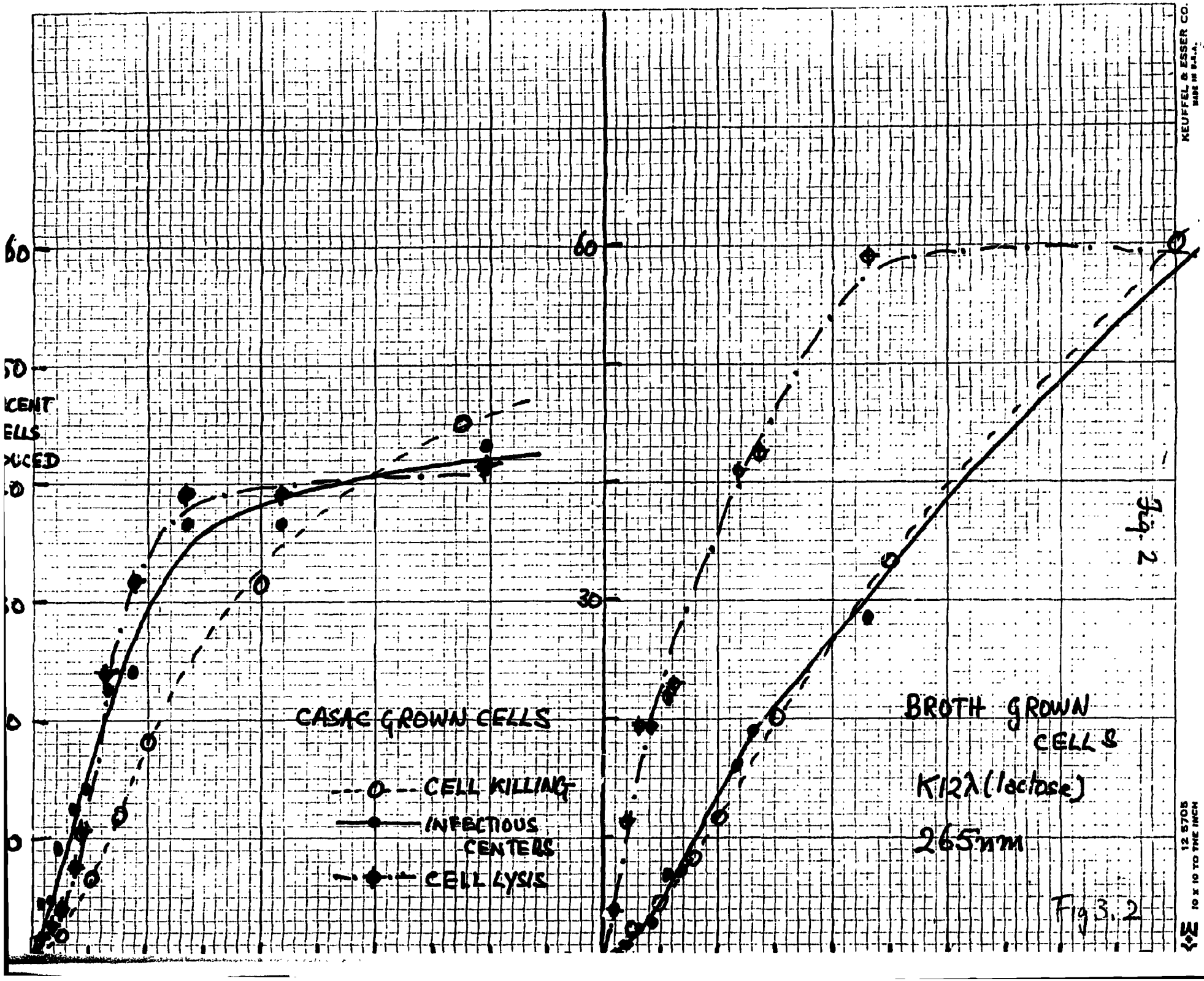

LYSIS
INFECTIOUS CENTERS
CELL KILLING
RCENT
LLS
DUCED
UV DOSE (J/m²)
K12λ(Hoshino)
280 nm
April 24, 25 1978
Debrot p 243, 244
with D.J.F.
Normalized to 41%

Fig. 1


60
50
KCENT
ELLS
XCED
0
30
0
0
60
30
CASAC GROWN CELLS
CELL KILLING
INFECTIOUS CENTERS
CELL LYSIS
BROTH GROWN CELLS
K12λ (lactose)
265nm
Fig. 2
Fig. 2
KEUFFEL & ESSER CO.
MADE IN U.S.A.
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10 X 10 TO THE INCH

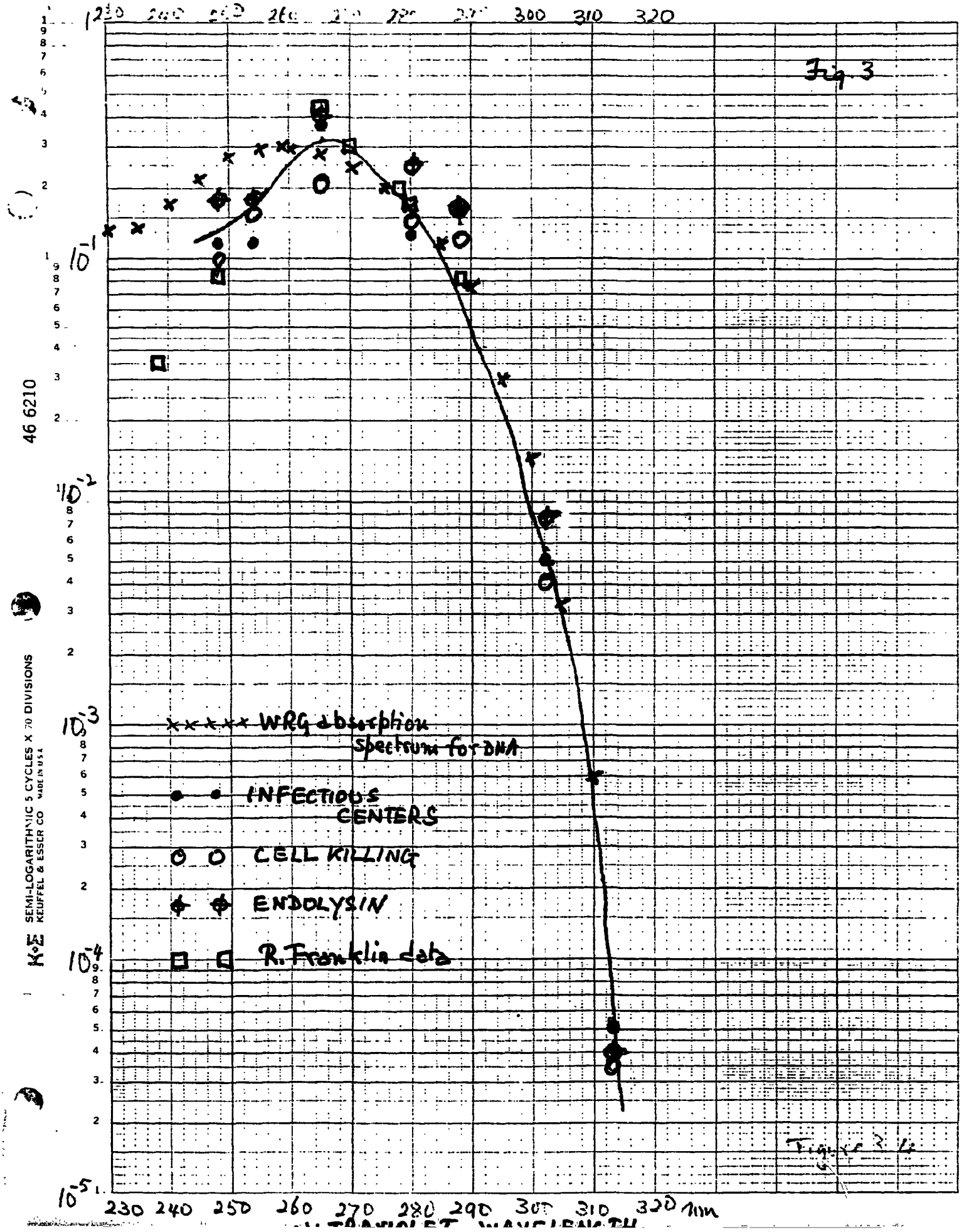
Fig 3
46 6210
SEMI-LOGARITHMIC 5 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO MADE IN U.S.A.
WRG absorption spectrum for DNA
INFECTIOUS CENTERS
CELL KILLING
ENDOLYSIN
R. Franklin data
230 240 250 260 270 280 290 300 310 320 nm

CHAPTER FIVE

Phenomena induced by damage to the DNA.

We have previously described three types of repair of radiation damage and the conclusion can fairly be drawn that if all three are missing the resulting cell must be very sensitive. Experience proves this to be quite right: if one has a mutant in recA and uvr and handles the cells out of the range of photoreactivating light, then one pyrimidine dimer per genome is lethal to the cell that depends on that genome. One can thus conclude that close study of these three processes will reveal all there is to know about the repair of radiation damage.

It has proved that this is a half-truth. There is an added factor, and the simplest way to give a mind-picture of the new factor is to remind the reader that all the three processes we have described depend on mechanisms which are either in the cell or not. If they are there then the repair process will go forward: if not, the cell is vulnerable. The new factor is initially not in the cell,at least to any important degree, but the effect of the right "insult", of which UV and IZR are representatives, is to set going a process which generates, in something close to the time for a cell division, an altered cell, with new reactions to the repair process. This development of a response is called an induced response and in this chapter it is our intent to give a summary view of the multiple aspects of this kind of induction as a preliminary to considering each in some detail.

Prophage and lysogens.

The earliest example of the induction of an altered cell by

agents which damage, or alter, the DNA came shortly after Lwoff discovered that bacterial viruses could be integrated into the bacterial chromosome and multiply exactly with that chromosome. Along with this was also the property that disturbances of various kinds, some spontaneous, could cause a change and the concordant multiplication could abruptly alter, resulting in the independent multiplication of the bacterial virus and the lysis of the original cell. The integrated, concordantly multiplying virus was called a prophage and the cell which had such a prophage a lysogen.

This system proved to be rather simple and sensitive to study. The spontaneous rate of change from lysogen to lytic is not great, and the result of one such change is at least one viral plaque on an indicator strain of bacteria. This is readily observ ed. So any agent which will increase the rate of passage from lysogen to lytic produces an experimentally readily detectable event. Thus it was not long before Lwoff, Simonovich and Kjeldgaard showed that ultraviolet light greatly increases the yield of phage. In fact, the appropriate dose of UV will cause the change in 100% of the cells. UV is then characterized as an inducer, and the process is called the induction of the prophage. At about the same time a considerable number of agents were found to be inducers also. IZR is one, nalidixic acid another, mitomycin C a third: all quite efficient, but all differing in some details: for example IZR only induces about 25% of the lysogens.

A great advance took place with the introduction of the K12 strain of E.coli, and the study of the prophage λ. We intend to describe this process in some detail in the next chapter, but we can mention that as the various mutants in E.coli strain K12 began to be characterized, it was possible to examine the lysogens of these strains. The characteristic of lysogenic phage such as λ is that upon infection of a normal host a small percentage of the infected cells become lysogens and now acquire an immunity

to further infection. Thus lysogens of the various mutants of K12 of interest can be constructed and then studied for induction. It was found that most of these mutants could be induced, though by no means equally as regards dose of the inducing agent, but that recA mutants could not be induced at all. A second mutation, lex, which generates a cell which is susceptible to post radiation DNA degradation, particularly if IZR is used, behaves in a strange way as regards inducing agents. It gives a normal yield of lytic phage for very small inducing doses, but as soon as a very small limit is reached, the yield falls drastically. Thus these two mutants have a definite effect on the inducibility of prophage in this system. Moreover, it does not matter much what agent is used to induce: there is no induction in recA$^-$ and a small spurt in lex$^-$.

The fact that UV is an inducer of λ makes it possible to vary the wavelength and see what is the maximum of effectiveness. This was done quite early by Franklin and the data strongly suggest that the absorption of UV by nucleic acid is the predominant event in the induction.

The discovery of prophage and of its induction by radiation and other agents was made quite early, in fact at about the same time as the discovery of the first radiation repair agent: photoreactivation. The stage was therefore not set for observation of the relationship between the induction of prophage and the phenomenon of induced repair. Nevertheless two important observations were made shortly after this initial discovery which are of considerable importance in work being done today. The first of these two was made by Weigle and the second by Ethel Tessman. They lead to the discussion of what we shall call W- (for Weigle) reactivation.

W-reactivation.

In 1953 J.J.Weigle discovered that ultraviolet irradiated phage λ, which would normally show a survival of only about one phage in 10^4

when assayed for plaque formation on an unirradiated host cell, showed considerably more survival if the host had first been subjected to ultraviolet irradiation. He went further in this interesting experiment. One characteristic of bacterial viruses is that of mutation and one rather drastic mutation of λ involves a change which produces a virulent strain with no fraction entering the lysogenic pathway. This mutation causes the normally cloudy and centered plaques of λ to become clear. It is not a common phenomenon, only about 1 in 10^5 plaques show the clear mutation. Weigle observed that the number of such clear plaques was much higher when the indicator bacteria had been subject to UV irradiation. Thus the effect of irradiating the host cell not only gave more survivors of UV damaged phage, but also these survivors contained more mutant phage. So some alteration in the host cell had occurred which favored the survival of phage and which also favored the occurrence of mutant phage.

In 1956 ~~Some years later~~ Ethel Tessman and Ozaki showed that the survival of UV irradiated T1 phage is higher on UV irradiated cells and that there is also a higher rate of mutation under those circumstances, thus adding to the generality of W-reactivation. Miura and Tomizawa in 1968 showed that both W-reactivation and W-mutagenesis require the recA+ gene product. This phenomenon of W-reactivation has been subjected to more recent study. We return to those findings later. It proves to be one of the most useful ways to study the process of induction. Defais, Fauquet, Radman and Errera, in 1971 showed that lexA+ is also required.

Filamentation.

One significant effect of UV irradiation is to produce division delay. This is associated with elongated cells and the observation of a UV irradiated culture in the microscope reveals that normally cells will grow up to four or five times the normal length before dividing. One strain of E.coli, strain B, one which had been used for many years as a normal strain, showed that upon being given very small doses of UV

very much longer forms were observed. These can be up to 100 normal cell lengths and they have been given the name of "filaments". It was natural to look for a mutation which was related to the formation of the material of the septum which separates two dividing bacteria and this genetic locus was found and identified. Its equivalent in strain K12 was found and named. Since a great deal of the genetic understanding of E.coli has been in strain K12 we give the name of the genetic locus found in that organism by Howard-Flanders, Simson and Theriot namely lon. A cell which is lon$^-$ lacks one or more of the enzymes necessary for the synthesis of the material of the septum and it will therefore not divide. Adler has made a considerable study of the factors which relate to filamentation and has shown that if extracts of non-filamenting cells are included in high concentration with filamenting cells which have received a dose of UV, the amount of filamentation and the concomitant survival of colony forming ability is much higher. So there is a biochemical reason for the lack of septum formation and it can be remedied by supplementation.

In considering the factors which influence filamentation Witkin was struck by the analogy to the induction of a prophage. The integration of viral DNA into the bacterial chromosome does not necessarily have to comprise the whole DNA necessary to generate a complete lytic phage. Defective prophages abound: these are prophages in the usual sense, but they lack some factors which will generate a whole virus particle: in strain 15 the head and tail can not combine. So, if one supposed that the filament former, in addition to carrying the lon$^-$ mutation also had an inducible characteristic, which Witkin associated with a prophage, then the nature of the induction process, including the agents which induce and the amounts of them (rather small) could be explained. Thus Witkin made the proposal that in addition to the need for genetic factors, filamentation is an inducible process. This proved to be very important, for filamentation is readily and almost immediately observable, and mutations in the induction process could be sought.

Also, Witkin made it clear that although filamentation might be associated with the induction of some kind of prophage, the two processes could have a common initial derepression, but separate final expressions. Thus the presence of a defective prophage might not be essential to the induction of filamentation.

Not long after this suggestion by Witkin an important advance was made by Kirby, Jacob and Goldthwait. They isolated a strain of K12, T44, which is a lysogen which is heat sensitive for the induction of λ-prophage. At temperatures above 40°C the prophage is induced. They also observed that in this strain filaments were formed at the elevated temperatures and that the normal modifiers of filamentation, including pantoyl lactone, which is effective in preventing filamentation, also prevented filamentation at the elevated temperatures. Adenine helped to promote filamentation and guanosine depressed it. Thus the experimental confirmation of Witkins suggestion was at hand and also a most useful mutant for study of induction processes.

Inducible Inhibition of post radiation DNA degradation.

In 1960 J.H.Stuy observed that several strains of bacteria showed the phenomenon that after ionizing radiation their DNA underwent considerable degradation to acid soluble fragments. Shortly after this, Miletic,Kucan, Draculic and Zajec showed that chloramphenicol increased the postradiation DNA degradation (a mouthful of words which we shall shorten to "prd"). A little later they also showed that a preliminary dose of IZR produced less prd when a second dose was given, a phenomenon independently observed by Pollard and Achey. These observations were put into the category of inducible phenomena by L.J.Grady, who showed that in E.coli strain 15, which normally harbors a defective prophage, the amount of prd depended on factors such as the medium (less in rich medium), on the presence of chloramphenicol, on amino acid starvation (more), while

a strain JG151 which had been cured of a colicinogenic factor and so presumably of the defective prophage, showed none of these differences. The amount of prd was uniformly high whatever the medium, whether chloramphenicol was present, or aminoacid starvation was used. In concomitant studies Pollard and Weller showed that the extensive cell lysis found when the defective prophage was induced was absent in strain JG151. So the suggestion was made that there is an inducible inhibitor of prd and that this is related, in somewhat the same way as filamentation, to the induction of a prophage.

It is of interest that one aspect of both filamentation and prophage induction which caught the attention of Witkin is the all-or-nothing response. A cell is either induced for λ or it is not: there is no in between. Once again this proves to be a half-truth, about which more will be said later, but the first broad conclusion is that prophage is either induced or not and so is filamentation.

In 1972 while studying whether prd is all-or-nothing, it was found by Pollard and Randall that if prd were looked for in a strain which carried the property of the inducible inhibitor, the inhibition was all-or-nothing. In other words, if the average prd were 50% then a large number of cells had <u>all</u> their DNA degraded while an equal number had their DNA essentially intact. This phenomenon was observed in a strain with no very noticeable prophage and it suggested that the inducible inhibition was not necessarily related to the presence of some kind of inducible episome. Accordingly the phenomenon was studied in terms of the time sequence of inhibition and it was found that about one division time was needed to reach the maximum expression. Induction could be achieved by several inducers, notably IZR and nalidixic acid as well as UV and a strain dependence was observed: there was no inhibition in strain B_{s-1}, which is <u>lex</u>$^-$. Two subsequent studies, by Marsden, Pollard, Ginoza and Randall and Pollard, Randall, Keller and Boyce showed first that the inhibition property

was absent in recA⁻ and lex⁻ strains and second that the presence of λ-prophage did not markedly alter the behavior of inducible inhibition of prd. Thus this phenomenon is closely like the others just described.

How the inhibition works is not yet known. The enzyme system most favored for the degradation process is exonuclease V, a three way nuclease, which is coded for by the two recombination mutants recB and recC. recB mutant cells which have been lysogenized for λ-prophage can be normally induced by UV. This suggests that the induction process is working in such a cell. There is no inhibition of the very small amount of prd which is observed in these cells, so it would seem that inhibition is somehow concerned with the effective functioning of this enzyme. However, direct proof of the inhibition has not yet been reported. The actual signal for prd is not yet known: it may involve a first attack enzyme which is the focal point of inhibition.

Protein X: the recA gene product.

The phenomenon of filamentation and its relation to the gentics of recA and lex suggested to Inouye that there should be an inducible protein molecule which would be involved in some way with the synthesis of the material needed for septum formation. He argued that such a protein should be membrane related and set out to look for it. He separated membrane bound proteins and looked at them with gel electrophoresis. He found a band which was present in UV irradiated normal cells, but not present in recA⁻ cells. This work was completed and published in 1971 and attracted remarkably little attention, probably because the idea of a coordinated set of phenomena related to radiation damage and the recA and lex genes was held by only a very few people. It is very interesting that as the idea that such a phenomenon was indeed to be found in cells gained support,

two laboratories quite independently rediscovered this induced protein, already named by Inouye "protein X". Indeed, it almost seems as though homo sapiens the examination of E.coli cell proteins by was intended to find this induced protein, for in SDS polyacrylamide gels there is a significant gap which lacks any band and in which protein X nicely appears. In addition to the rediscovery independently, further work by Gudas and Pardee in the same laboratory as Inouye considerably extended the findings.

It soon became apparent that strains of cells which are recA$^-$ and lex$^-$ show very little protein X. In the case of recA$^-$ it is adequate to say that there is none. The lex$^-$ case is not so absolute. One can get small amounts of protein X with inducing agents, but the amount is always small and hard to demonstrate. So long as experiments are conducted with wild type cells and compared with recA$^-$ cells then all the inducing agents that are associated with the induction of λ and the other phenomena we have described, function effectively to induce protein X. When various classes of mutants are examined it becomes possible to see that some inducing agents will induce protein X and others not. Thus a recB mutant can be induced by UV or bleomycin, but not by nalidixic acid. It was also found that some strains of cells existed which had protein X before any induction process. One of these, isolated in the authors laboratory by Horan and Hird, for the criterion of non degradability of the DNA after IZR, strain PHH1, proved to have high constitutive levels of protein X. It is also very insensitive to ionizing radiation and undergoes very little DNA degradation after that insult.

The induction of protein X after the inducing event is rapid: it is present in the cells within a few minutes. It is also produced unevenly in the cell cycle, being greatest when DNA synthesis is greatest. Removal of the inducing agent slows the rate of synthesis of protein X. The presence of protein X after induction continues for about

two or three division times.

Since there is a clear association between the presence of recA$^+$ and the inducibility of protein X, it is an obvious suggestion that protein X is the gene product of recA. However, for reasons discussed later this suggestion did not seem to fit all the genetic facts about induction and cell behavior. A scheme whereby a gene tif was responsible for the messenger RNA for protein X was proposed and recA and lex were assigned regulatory functions on tif. One of the most interesting recent developments has been the convincing demonstration in four different ways from four different laboratories that protein X is,in fact, the recA gene product, and moreover protein X exerts a regulatory influence on recA itself. The way in which this evidence has accumulated will be discussed much more fully later.

Thermosensitive Induction: the tif gene.

We have already mentioned that Kirby, Jacob and Goldthwait isolated from strain C600 a mutant which was a filament former if kept at 40^{o}C in the presence of adenine and also induced the lysogen for λ under those conditions. This mutant was further characterized in two important publications by Castellazi, George and Buttin. They were able to show that the gene carrying this property mapped extremely close to recA and that the thermal induction of the two phenomena studied previously was not all. They found that both W-reactivation and W-mutagenesis are expressed by the elevated temperature treatment. However, the phenomenon of induction by conjugation with a UV damaged donor bacterium did not occur if the donor bacteium was thermally induced. Thus no drastic change in the DNA structure was to be inferred. They further found a mutation zab again very closely linked to recA and which had properties suggestive of

available a very useful means of testing the effects of induction. Cells grown at 40+ degrees in the presence of adenine for 40 minutes or so were to be presumed "turned on" or induced, and now other effects which are dependent on induction could be looked for. For example, protein X is found in larger amount in the induced cells than in those kept at the permissive temperature of 30^{0}C or so.

Induction and respiration.

If one looks at the effect of UV or IZR on the consumption of oxygen by E.coli, the major first impression is that considerable doses are needed and that what effects are seen occur very much later than the application of the radiation. It is, however, found that there is a strain dependence of these effects and they also are different in different media. In 1970 Swenson and Schenley observed that E.coli cells grown in minimal medium with glycerol as a carbon source showed a cessation of respiration after UV treatment. Thecessation began at about 70 minutes after the treatment and was less if photoreactivating light was given after treatment or if the medium was supplemented with casamino acids. If chloramphenicol was given shortly after treatment the respiration deficiency was very much less. They began to consider seriously the idea that the loss of respiration is due to a radiation induced process. Looking at the appropriate mutants they found that the phenomenon takes place at lowest UV doses in uvr^{-} cells and it is nearly abolished in $recA^{-}$ and lex^{-} cells. Thus the proper behavior for radiation inducible phenomena is seen.

The question arises as to why the effect is seen primarily in cells grown on carbon sources like glycerol. Swenson looked at the analogous situation for the induction of β-galactosidase by lactose or analogs of lactose. In the presence of glucose there is catabolite repression due to the depressed level of cyclic AMP in the cell in the presence of glucose.

The addition of cyclic AMP to the cells increases the induction of β-galactosidase by lactose. He found that the respiration reduction after UV was strikingly greater in the presence of cyclic AMP.

Swenson has further made correlations between the induced respiration cessation and the death of cells and has gone far in showing that non-viable cells after UV induction in this way, are those which are induced for respiration stoppage.

Induced UV mutagenesis.

Of great, and perhaps central importance in this whole area of work is the demonstration of induced UV mutagenesis. The first evidence for this was in the work of Weigle and Ethel Tessman which we have already mentioned. The UV irradiation of the cell to be infected by UV irradiated λ or T1 phage produced more mutant phage in the resulting progeny. In the case of mutations in the cells themselves the effect of ultraviolet light was found very often to give a mutation response which was not proportional to the UV dose but very much more like the dose squared. For example if leucine revertants are studied as produced by UV exposure, there is a rapid increase in the number of revertants per survivor as the dose goes up to a value where cell death begins to be appreciable. This type of behavior suggests that there is a dual necessity for mutagenesis: there needs to be the mutagenic lesion, but in addition there needs to be something, also dose dependent, which acts on the the mutagenic lesion to produce the mutation.

The first most convincing demonstration of induced UV mutagenesis was given by Witkin using the tif system. This will be discussed much more fully later and for now we can say that in this strain 100% induction can be produced by holding the cells for 40 minutes in the presence of adenine. Witkin found that if she gave ultraviolet light and kept the cells at 30°C 30

[redacted]

that the induction of the cells did not occur, the yield of mutants followed the normal dose squared response. However, if the temperature was raised to 42°C in the presence of adenine for 40 minutes, the yield of mutants was increased and, moreover, they now followed a linear, not a squared relation with the dose. This strongly implied that the temperature induction had brought the cells into a new condition in which the only burden on the UV light was that of providing the mutagenic lesion and not, in addition, of inducing the cells.

Induced Radioresistance.

The induction phenomena we have so far described suggest that some kind of change in the DNA elicits an altered cell which can behave differently in many respects. Among these changes is filamentation and the inhibition of post radiation DNA degradation. The former might be thought to be adverse to colony survival and the latter possibly favorable, although recB and recC mutants which essentially do not undergo prd have less survival potential.

Early attempts to show that induced cells had increased survival value were unfortunate. However, the realization that the stresses applied to cells to test their ability to retain colony forming ability were also of the nature which induced the cells, suggested that to test increased resistance transcription should be blocked in the cells under test, after induction. This approach was taken by Pollard and Achey, who followed the routine of giving an inducing treatment of UV or IZR or nalidixic acid, permitting transcription and translation to occur by incubating for 40 minutes or so, and then applying the challenge stress, which was mostly IZR (X- or γ-rays) This technique revealed, without

any question that the induced cells were more resistant, providing they were recA$^+$, lex$^+$, and recBC$^+$. Independently of this type of experiment a very interesting study was made by Trgovcevich and Rupp. They took advantage of the properties of the thermally inducible strain of λ prophage. This is inducible because the repression system has allosterically changable repressors which do not repress at the non-permissive temperature. Thus it is possible to start the induction of λ and to stop it by a temperature jump applied over early induction times. Doing this they were able to show that the abortive, but initiated, induction of λ not only did not kill the cells, but rendered them considerably resistant to X-rays. This again did not happen in strains which were recA$^-$ or lex$^-$.

An interesting, and again independent, approach was taken by Smith and Martignoni. They employed x-rays as the means of induction and tested the sensitivity of cells against UV. Included in their procedures was the use of chloramphenicol to block protein synthesis. They also looked at the various mutants and reached somewhat the same conclusion as Pollard and Achey, with the feeling that the phenomenon may be more complex and not solely involved with the induction process.

Induced Repair in Mammalian Cells.

In spite of the rapid development in the understanding and what might be called the technology of the study of mammalian cells there is only one of the above phenomena which has been demonstrated convincingly in mammalian cells. This is what we have called W-reactivation. Bockstahler and Lytle showed in 1971 that the survival of UV irradiated herpes virus was considerably greater on UV irradiated host cells. Not all host cells gave the same increased yield. The reactivation needed time to develop and is inhibited by inhibitors of protein synthesis. It seems, as will be later

discussed that there is considerably more complexity to work with mammalian cells, or indeed any eukaryotic cells than there is with a well characterized strain of E.coli. A cell with mitochondria, chloroplasts and other organelles is almost certainly one which has evolved by the symbiosis of several originating kinds of cell. One interesting discovery to be predicted is that unless induced repair proves to be quite universal, it may well be manifested in parts of mammalian cells and not in all.

It will take considerably longer to establish the true extent of induced repair in human cells than it did in E.coli, where it took 20 years and is not yet fully established. The reason is in the power of understood genetics. In E.coli the understanding of the rec mutants makes it possible to test some new phenomenon against whether it shows in a recA$^-$ strain, for example. This can not yet be done in mammalian cells .

Conclusion.

This chapter has been intended as a summary overview of the subject of induced repair. It is intended as a kind of guide to the broad nature of the topic and its purpose is to set the stage for careful consideration of the various phenomena of induced repair separately. In doing this we intend to attempt some historical account of the development of the more significant indications of the existence of induced repair, as well as trying to give the current position.

SUMMARY OF RESEARCH

For the immediate future, given the opportunity of collaboration of E. C. Pollard and D. J. Fluke in the latter's lab at Duke, our chief focus is on induced repair. Beyond recognized levels of photoenzymatic repair, excision repair, and recombination repair, lies this fourth level of repair. Also called SOS or error-prone repair, and accessible chiefly in bacteria so far, the chief distinguishing feature of the process is its inducibility. Absolutely dependent upon the recA gene product and partially upon the lex gene product, this repair process develops incubatively in cells which have sustained some triggering insult or condition, including ionizing and ultraviolet radiations.

A chief practical interest in this process, beyond its obviously intimate role in cell biology, is its involvement in mutagenesis. A repair process which makes mistakes is of high interest in genetic hazards of radiations and indeed of other environmental mutagens and carcinogens.

We see our current and imminent involvement in this area of study as falling under five general questions. These can be stated as follows, with comment about our findings to date, our ideas, and our capabilities.

1) How general a phenomenon is induced repair?

We have one clear immediate opportunity to test generality beyond procaryotes. We plan a collaboration with the N. W. Gillham and J. E. Boynton group to look for induced repair in Chlamydomonas reinhardii, a well-studied eucaryote showing both Mendelian and uniparental inheritance. Both nuclear and organelle inductions can be studied. Some past work with this organism is suggestive of induced repair in relation to uniparental inheritance.

2) How single a phenomenon is induced repair?

(Can we separate the inductions of various manifestations of induced repair, or are they coordinate? For example, can we induce λ-phage separately from arrest of cell division, or induced radiation resistance separately from the induced inhibition of post-irradiation DNA degradation?) We can study this question better than most groups and are deeply involved in doing so. We have two studies already published which include tests of coordinate induction. In addition to precise, quantitative exposures to 265-nm UV at high intensity, and a wide range of capabilities for exposure wavelength dependence differences, we have ionizing radiation inductions and substantive damage exposures under accurate control.

3) How is the induction process related to post-induction cell division?

(How long does the induced state persist; to what extent is division modified by it, and vice versa?) We have good capabilities in this intricate area, and have made a start.

4) At the level of chemistry, what is the signal or agent to induce?

In this rather uncertain area of work we are thinking about examining the intact, folded genome of E. coli to see if a detectable change precedes

induction. In so specific a question, at the chemical level, intuitive approaches of this type are the only option we see for a positive lead toward chemical description.

5) What mechanisms are involved in induction?

(For example, proteases on λ-repressors, inhibition of the editing function of polymerase I?) This question will generally involve more biochemical manipulation than is directly within our capabilities. We consider it an area of possibility for specific opportunities that may arise and be practical for us as we go along. We are especially interested in close attention to such work in other labs.

SPECIFIC RECENT COMPLETED WORK

The paper in Radiation Research, 72:519-532, 1977, by Pollard, Person, Rader and Fluke, sets the stage for one of our main thrusts at present. Here we approach the problem of coordinate induction of mutagenesis, inhibition of post radiation DNA degradation, and induced radioresistance. We show that in one strain of cells the assumption that mutagenesis by UV involves both an inducing signal and a mutagenic lesion leads to an analysis of our findings which suggests that induction is coordinate, or nearly so.

Since this work was done in strain B, not normally lysogenic for λ, and since we had some preliminary findings that prophage induction can be exceptional, we changed our work to study strain AB1157, which can readily be lysogenized. In this we studied the induction of λ, induced radioresistance, W-reactivation, induced inhibition of post radiation DNA degradation. If broad terms are allowed, the three latter manifestations are coordinate, but the induction of λ is not, being more sigmoidal with dose and thus requiring more dose for effective induction. We are continuing this work which has a preliminary publication in the proceedings of the Keystone Conference on DNA repair, where it was reported.

We report, at the Toronto meeting of the Radiation Research Society, that W-reactivation can be seen for λ-phage irradiated with ionizing radiation which recover more of their plaque forming ability when infected into UV-irradiated host cells. Since ionizing radiation does not produce many pyrimidine dimers, if any, this shows that the induced repair system is responsive to damage other than dimers.

At the Burlington meeting of the Society for Photobiology we will report on two findings. One is a preliminary report of work done on the wavelength dependence of UV mutagenesis. This work enables some distinction to be made between the mutagenic lesion, the inducing signal, the damage to the irradiated cell, and the presence of some mutagenic process which does not conform to the induction/mutagenic lesion concept. The broad findings of wavelength dependence are to be discussed. In addition, work begun at Penn. State University by Mildred Rader, in which the effect of UV light given in the -60° frozen state is observed, is being reported. This exposure is thought to produce a high proportion of DNA-protein crosslinks, which are not photoreactivable. This component is an excellent inducer of the induced repair system and also is effective mutagenically. There is some evidence

that mutations produced are not in response to the induced repair system.

Not yet presented for publication but in quite good condition to discuss is work done by an undergraduate student, Brian Robertson. He has looked at the induction of filamentous growth in three strains: AB1157, which does not, properly speaking, produce filaments, but does elongate; AB1899, which is uvr$^+$ lon$^-$; and AB1896, which is uvr$^-$ and lon$^-$. He has plotted dose response induction curves for these three cells. He finds that the induction of elongation in AB1157 requires more dose than the induction of filamentation in AB1899, perhaps double, but that quite surprisingly, the introduction of the uvr$^-$ genotype does not alter the dose-response, other than to produce a higher number of apparently killed cells. This is a surprise, for the lon mutation is supposedly concerned with the biochemical pathways for septum formation. Yet it alters the dose-response for induction to almost the same degree as the uvr$^-$ genotype, which is concerned with the failure to excise dimers and the consequent formation of daughter strand gaps. Why the lon genotype should be equivalent to the uvr genotype in dose-response relation is very intriguing, and it suggests that somewhere in the induction process the cell membrane relationship to DNA may be involved.

PUBLICATIONS IN PROCESS

Three papers are in the accepted but not printed state.

1. Pollard and Fluke: Induction of radioresistance in four strains of E. coli; two of them λ lysogens. Biophysical Journal (page proof now being read).

2. Pollard and Fugate: Relative rates of repair of single-strand breaks and post irradiation DNA degradation in normal and induced cells of E. coli. Biophysical Journal. Just completed refereeing: minor changes for acceptance.

3. Fluke and Pollard: Dose-response relations for UV-induced repair phenomena. Accepted for the Keystone Conference Proceedings.

4. Volume II of "Contemporary Biophysics" Dekker. Radiation-Induced Repair. Pollard and Bockrath. This is intended to be a definitive statement of the whole phenomenon of induced repair. The first draft is 40% complete.

RESEARCH POTENTIAL AND PLANS

The melding of two separate but related research programs around the theme of radiation induced repair is clearly being productive. A good deal of preliminary work, not suitable for reporting here, has already been done: for example, preliminary action spectra for induction, mutagenesis, W-reactivation and bacterial killing have been plotted. With this kind of background we can push the productive lines of study. This is being done. We have an excellent monochromator, good radiation facilities and the needed equipment for most of the induced repair studies. We have good absolute dosimetry on all our radiation studies. If it works out, we expect to add a faculty leave-of-absence member, already experienced in the subject of induced repair. Negotiations are underway for this appointment. We expect to have two outstanding undergraduate students helping us in September. Experienced summer help is expected to be available.

GENERAL SIGNIFICANCE OF THE WORK

This work will shed light on the processes of mutagenesis and carcinogenesis. The first is involved with the correction of errors of insertion of bases into the DNA in some way, for which hypotheses exist, not in place to discuss here as they are not as yet confirmed by experiment. It is quite clear that induced repair plays some part in this operation. The relationship to carcinogenesis is not as definite. It now seems that chemical carcinogens cause alterations in the DNA of cells, and that whether they become transformed cells may well depend on the repair systems which operate on the changed DNA. One of these repair systems, now known to be involved in crosslink repair, long patch repair, and double strand break repair is the induced repair system we are studying. It would be very unwise to suggest that this induced repair system is without influence on the removal of potentially carcinogenic DNA damage. Indeed, enthusiasts can be found who will say that it is the induced cells which start the cancer. We are more conservative than that.

One area of direct interest to the Department of Energy is that of formulating guidelines for the safety regulation of radiation producing facilities from power reactors to diagnostic X-rays and various kinds of light. We have been involved in this area for a number of years. To us the advent of the findings of induced repair brings a potential for understanding of many of the seemingly complex dose-response findings of past, more empirical, studies: for example split dose findings. We feel that the proper understanding and knowledge of the scope of this induced repair phenomenon will be of the utmost use in setting safety regulation for doses of radiation which are far too small for statistical success in study on animals, yet which may be potentially harmful, over the years, to human beings.

DOSE RESPONSE RELATIONS FOR UV INDUCED REPAIR PHENOMENA[1]

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ABSTRACT Dose responses for four UV-induced phenomena have been examined for induction with monochromatic UV light at 265nm, using the same irradiation techniques and dosimetry. These show that for W-reactivation of UV-damaged λ-phage, induced inhibition of post radiation DNA degradation and induced radioresistance the relations are similar, though not identical. In the case of the induction of λ-prophage the relation is clearly different, sigmoidal rather than sharply rising at low doses. Inactivation of the λ-repressors evidently involves some additional dose-dependent process.

Introduction. Manifestations of induced repair of DNA include the inductions of λ-prophage, of Weigle (W) reactivation and mutagenesis, and of filamentation, induced radioresistance (irr), induced mutagenic action, induced inhibition of post radiation DNA degradation (prd), and induced respiration halt (1). All of these processes are thought to follow from the derepression of the recA gene, with appearance of relevant cell proteins (2,3). If all processes follow from one such event, then the inductions by ultraviolet light (UV) should be closely related, as coordinate inductions. In strain E.coli AB1157 we conclude that coordinate induction is approximately observed for W-reactivation, induced inhibition of prd, and irr, but not for the induction of λ-prophage.

W-reactivation. To avoid storage changes we prepared λ-phage weekly, by 60J/m^2 254nm UV exposure of AB1157λ cultures growing in Vycor flasks. After two hours subsequent incubation, which cleared the cultures, such "inductates" were further irradiated by 3,000J/m^2 exposure at 254nm, in the same flask. Parallel dilution of unirradiated inductate was made to the irradiated plaque survival level, ca 10^{-5}. For test of W-reactivation these phage preparations, irradiated or diluted control, were adsorbed to AB1157 cells in attachment medium (0.01M $MgSO_4$ in 0.01M TRIS-HCl), after these host cells had been grown in maltose, C-minimal salts + casamino acids.

[1] This work was supported by DOE Contract EY-76-S-05-3631.

Phage attachment was corrected for free phage by titer of some supernatants, after plating complexes at 10,15, or 20 minutes attachment time. E.coli Q1 was the indicator strain.

W-reactivation was tested by survival of the irradiated phage complexed with host cells irradiated at 265nm, in the attachment medium. Cell irradiations were made using a double water-prism monochromator, with absolute dosimetry and intensity averaging. Numerical considerations for W-reactivation were: unirradiated phage titer n_o before dilution, irradiated phage titer n for unirradiated cells after UV dose D to the phage, and irradiated phage titer N after dose d to the host cells. With sensitivity cross sections S and W(d) for the UV-phage on unirradiated and irradiated cells respectively, an appropriate measure of W-reactivation is:

$$\frac{S - W(d)}{S} = \frac{\ln(N/n)}{\ln(n_o/n)}$$

Such expressions of data from three experiments are combined in Figurel. The dose-response increases sharply at low doses to the cells, with a maximum S - W(d) about 0.25.

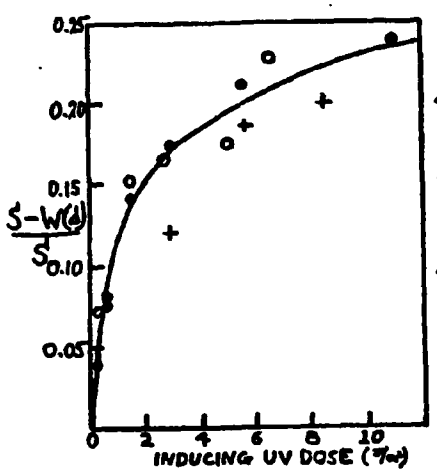

Figure 1. W-reactivation

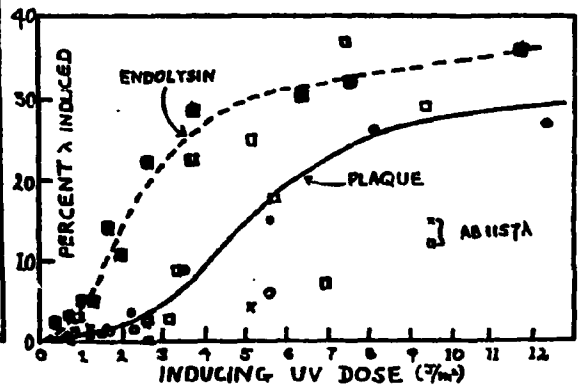

Figure 2. Two methods of observing λ-induction.

The induction of λ-prophage. One of two methods used to study the induction of λ-prophage involved plaque formation. Infectious centers were observed at zero time of incubation and also the yield after burst. For AB1157λ we found the results from the infectious center assay the more variable, though not in conflict with the full burst findings. Strain K12λ, which contains no difference in radiation-involved genes, was more consistent in the infectious center assay. Since it, unlike AB1157 is not lac⁻, a second way of observing the induction of prophage was possible. As used by Coetzee and Pollard (4), in cells grown on lactose and therefore induced for

β-galactosidase, the cellular enzyme is unable to reach the indicator substrate ONPG while the cell wall is intact. Induction of cell wall endolysin can provide a colorimetric index of the number of cells which have been induced. The results of both methods are shown in Figure 2, mostly for strain K12λ, but in good agreement with observations on AB1157.

The two methods of observation are not in agreement, although both show a sigmoidal response, in which the most rapid increase occurs at $2J/m^2$ for the endolysin assay and $8J/m^2$ for the plaque assay.

Inducible inhibition of post radiation DNA degradation. Cells are labeled with ^{3}H-thymidine, given various inducing doses, incubated for 50 minutes at 37°C, given 50μg/ml rifampin to block further induction and then X-rayed (31krad). Untreated cells show about 75% DNA degradation to acid soluble fragments. After various inducing doses of UV and incubation, cells show less DNA degradation, dependent on dose. The fractions of inhibition estimated from such data are quite similar, except for the dose range, to previous reports for strain WU3610-89. (5). The findings, averaged from three experiments are shown in Figure 3, without data points. The inhibition rises sharply at low doses, with dose-response relationship more like that observed for W-reactivation than for the plaque assay for the induction of K12λ.

Induced radioresistance. Cells are given pre-treatments with 265nm light, incubated for 50 minutes at 37°C, given rifampin and then given a series of X-ray doses to enable a

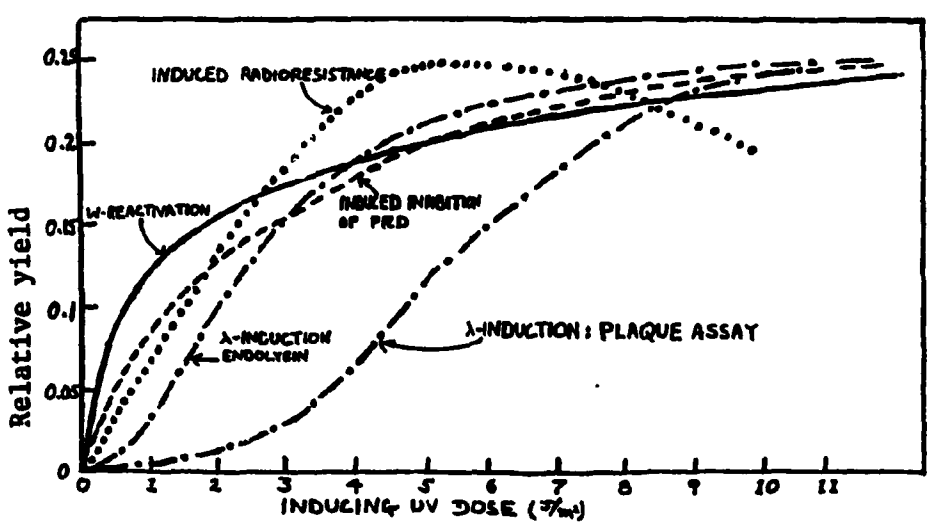

Figure 3. Comparison of the four processes studied. The sigmoidal behavior of λ-induction is in contrast to the initial sharp upward slope of the others.

survival curve to be plotted. Pretreated cells are more X-ray resistant, depending on the UV dose. The amount of resistance so induced was determined by reconstruction experiments, mixing unirradiated and fully induced cells in various proportions. A resulting calibration curve was used to estimate the fraction induced for each UV dose. The findings appear in Figure 3.

Discussion. Figure 3 summarizes the results. Adjustment of all have been made to the 0.25 maximum for W-reactivation. While no pair of induction dose-response curves are closely identical, there is similarity in shape for the curves for W-reactivation, induced inhibition of prd and induced radioresistance. In contrast, the form of the curves for λ-induction is different. The endolysin assay is in rough agreement with the early findings of Marcovich (6) who showed a dose-squared UV induction of λ in his system. Our plaque assay deviates from this relation at the lowest doses.

Previous report on strain WU3610-89 (5) has shown that induced inhibition of prd and UV induced mutagenesis can be brought into coordinate relation if UV light acts in one instance to cause premutational lesions and separately to induce a process converting these to mutations. Induced radioresistance, while not identical, is similar in behavior. The four processes studied here seem to group into 3 which are probably simply related and the fourth, the induction of λ, which is more complex. We feel that the induction of λ is probably also a two-step process in which UV action is needed at least twice.

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UV Induction of Filamentation in E. Coli: A Dosimetric Study of uvr and lon Strains

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Zoology 192.52

In recent years, the phenomenon of bacterial filamentation has received attention as one of the many ramifications of the induced repair system. Filamentation-elongation reflects a defect in septum formation which is believed to be a consequence of the induced (rec A) products. The lon^- strains are especially sensitive to these products and produce nonseptate multinucleate filaments which are many times the length of uninduced cells. Research in filament recovery (Kantor), the relationship between induced products and septation (James), and induction-dose effects (Kantor) indicates that filamentation is an accessible approach to the study of induced repair. Most recently, the induction-UV dose relationship explored by Pollard and Fluke calls for similar research in filamentation.

Clearly, a study of the effect of induction on the lon strains is essential for an understanding of the nature of filamentation. On the other hand, the uvr strains (due to the lack of excision repair) have been a classical tool for the study of induced phenomena. Combining aspects of filament sensitivity (lon strains) with induced repair sensitivity (uvr strains) forms an effective basis for the study of the relationship between these two phenomena. This research consists largely of a comparative study of UV induction of filamentation in the four possible combinations of these strains (AB 1157--uvr^+ lon^+, AB 1899--uvr^+ lon^-, AB 1886--uvr^- lon^+, and AB 1896--uvr^- lon^-).

Materials and Methods

All cultures were grown on Robert's C-minimal salts supplemented with two mg/ml Casamino acids and five mg/ml glucose as

a carbon source. The cells were incubated at 37 C until they reached a concentration of about 10^8 cells/ml and then iced. The cells were irradiated under 265 nm monochromatic light using a water prism monochrometer. The cells were irradiated in eight ml quantities in a 46 mm fused silica dish. Three doses were given additively to each dish--extracting the dose sample in 2.5 ml quantities after each successive dose. One-half ml of this sample was then transferred to a dilution blank for a survival analysis of the immediate effects of the UV light. The remaining two ml quantity was incubated at 37 C for 90 min. After irradiation, these operations were carried out under yellow light to prevent photoreactivation. The entire procedure was carried out for six to eight dose samples per strain (including the control--no irradiation).

After the 90 min incubation, the cells were iced and a second post-incubation plating sample (.5 ml) was removed from each dose sample. A very small drop of the iced sample was then placed directly on the grid region of a Petroff-Hauser Bacterial Counter and firmly covered with the cover slip. (Large drops result in mass migration due to unequal pressure on the cover slip.) Cells were then examined under high and dry magnification and appeared negative (white) in contrast. Photographs of the bacteria were taken using 35 mm Panatomic X film and a one second exposure on a Leica camera attachment for the microscope. At least five photos were taken of each dose sample at different locations on the grid to insure a representative distribution. Only the central eight squares of the grid appear in the photos (i.e. the field

of view for the camera is about half of that which is seen through the ocular).

The appropriate light for the exposure time was obtained, first, by setting the light source control on "high." Second, the condenser was positioned by focusing the low power objective on a stage slide or transparent ruler and bringing the condenser glass into focus using the substage condenser focus. Then, the low power objective was focused on the iris of the diaphragm and the diaphragm diameter adjusted to match the diameter of the field of vision.

Iced cells tend to be less motile and photos were taken as quickly as possible to minimize stage heating. A one second exposure is too great to allow for substantial mass drift or motility. Film and prints were processed with Kodak developer D-76 and prints were made on Kodabrome HF RC paper. Pictures were analyzed by the measurement of individual cell length in millimeters using a flexible ruler to accommodate filament shape. Results were tabulated by length in millimeters and UV dose. Survival samples were plated in .5 ml quantities on 15 mls of nutrient agar in dilutions from 10^{-2} to 10^{-7}.

Results and Discussion

Time rate of development. Ninety minutes was chosen as the incubation period as a result of time rate of development for filamentation and survival experiments on AB 1157 and AB 1899. The 90 minute period is a compromise between the extensive amount of time required for the formation of obvious filaments (particularly in the lon^+ strains)--four doubling times, and the

least amount of recovery from filamentation--about 60 minutes. (After 60 minutes, the number of normal cells begins to increase relative to the number of filaments--thus, decreasing the total filament ratio.) Figures 1. and 2. illustrate these points for the AB 1899 strain. Similar relationships apply to all four strains.

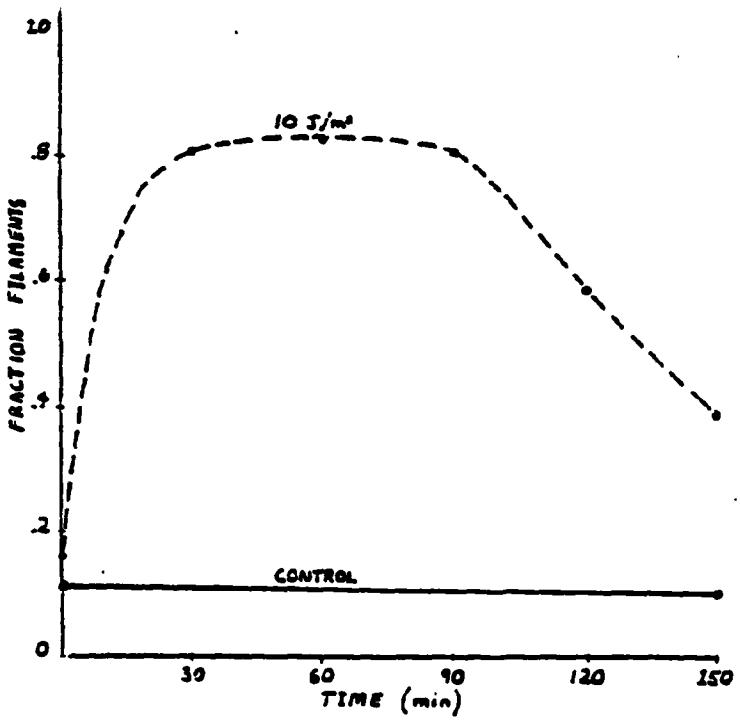


FIG. 1. Time rate of development for filamentation in AB 1899. Symbols: o, control; •, 10J/m². Note that peak filamentation occurs at 60 min and drops off progressively with time.

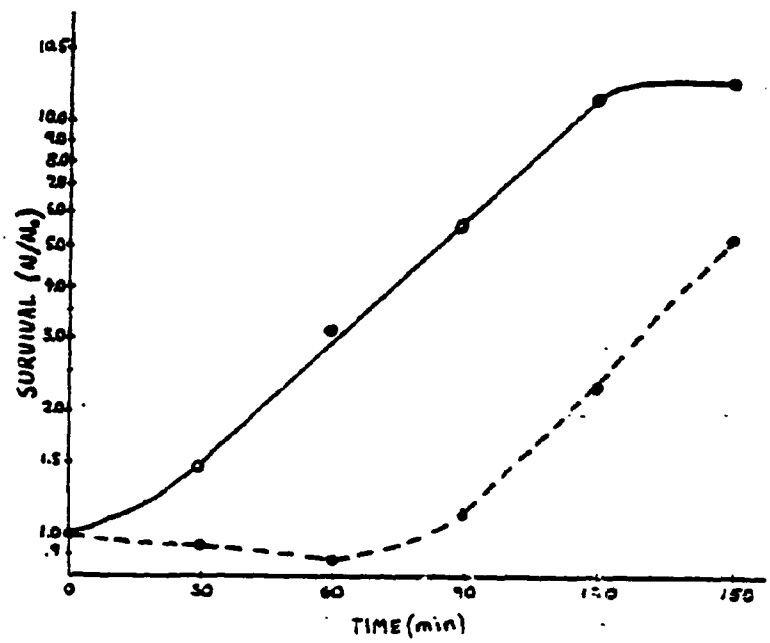


FIG. 2. Time rate changes in survival (colony formation) in AB1899. Symbols: o, control; •, 10J/m². UV produces a decrease in colony formation up to 60 min whereupon cells show recovery.

What length characterizes an induced cell? Figure 3 indicates the induction of the shortest length classes (two to five millimeters as measured from 5:8 inch enlargements) as a function of UV dose. Clearly, the first class to be induced (i.e. to show an increase in ratio with increasing dose) is the five millimeter class. In all analyses, this criterion forms the basis for determining the cell length which represents

the minimum length for induction.

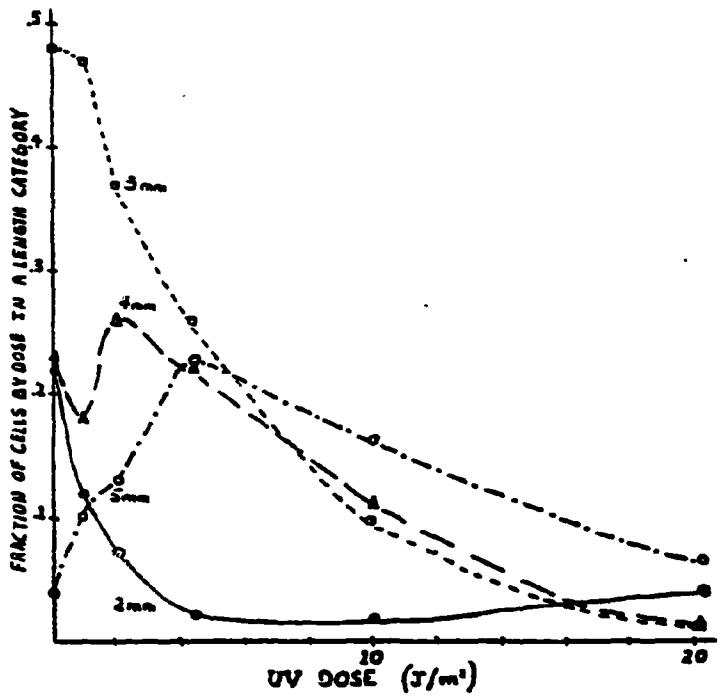


FIG. 3. Induction of length categories vs. UV dose for AB 1157. Symbols: ●, 2 mm; ▲, 3mm; ▲, 4mm; ○, 5mm. Note that 5mm is the first length class to show induction with increasing UV dose. Thus, 5mm is designated the base length for filamentation-elongation.

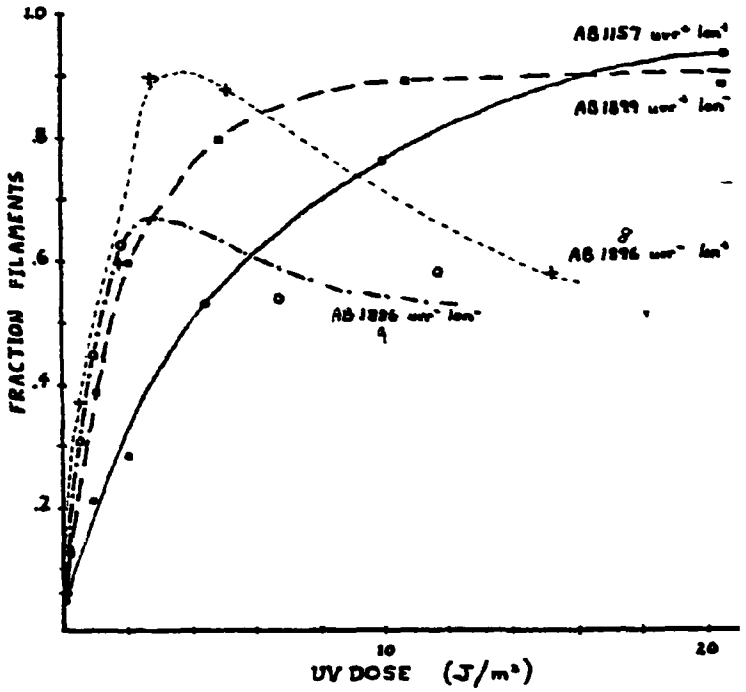


FIG. 4. Induction of filamentation vs. UV dose for all four strains. Symbols: ●, AB 1157; ▲, AB 1899; +, AB 1886; ○, AB 1896. Decrease in the slope of the uvr⁻ strains may be attributed to sensitivity to higher UV doses resulting in cell death before filamentation.

Induction curve. Figure 4. shows the induction (filamented or elongated cells over total cells) versus UV dose for the four strains. Table A. contains a more readable index of the induction dose relationships of Figure 4. The table indicates the fraction of all cells induced at 2.0 J/m^2 (simply read from the graph) for the four strains. Two J/m^2 is a convenient dose which corresponds to the ascending portion of the curves so as to minimize the effect of higher dose UV sensitivity in the uvr$^-$ strains. The table indicates the relative "steepness" of the curves.

	lon$^+$	lon$^-$
uvr$^+$	.31	.60
uvr$^-$	.70	.65

Table A. Fraction of cells induced at 2.0 J/m^2.

Survival. Table B. shows the corresponding post-incubation survival ratio relative to the control (N/N_o) for these same strains at 2.0 J/m^2. A comparison of Tables A. and B. reveals the relationship between cell death and filamentation. This relationship is significant in qualifying the filament ratios, since extensive UV-induced cell death before filamentation would drastically reduce the filament ratio.

	lon^+	lon^-
uvr^+	.58	.55
uvr^-	.18	.09

Table B. Colony formation relative to control (N/N_o) at 2.0 J/m^2 (post-incubation).

Size distribution. When the fraction of cells in a length category over all cells for a particular dose is plotted against the UV dose, cell lengths tend to cluster into groups or peaks about 2.5 millimeters apart for the strongly inducing doses. Table C. shows the "strength" of the distribution of induced cells into length classes for the four strains. The index of distribution strength represents the number of peaks in which the fraction of cells of a filament length (≥5 mm) accounted for 10% or more of all the cells for a particular dose, totalled for all the doses.

	lon^+	lon^-
uvr^+	6	8
uvr^-	5	3

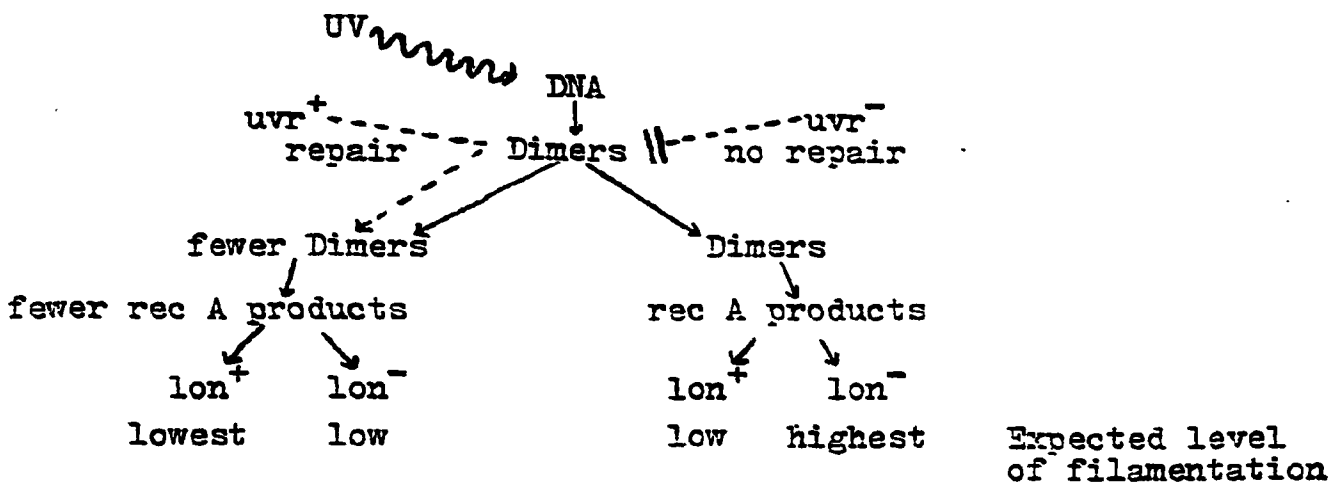
Table C. Index of distribution strength (see text) totalled for all the doses for each strain.

The following points follow from Tables A., B., and C.:

- All four strains show a significant induced effect.
- uvr^- enhances the induction of lon (especially lon^+) relative to uvr^+.
- lon^- induction is not as strongly dependent on the uvr genotype as is lon^+.
- uvr^- lon^+ shows maximum induction.
- uvr^+ lon^+ shows minimum induction.
- Survival roughly negatively correlates filamentation (greater filamentation yields lower survival rates).
- lon shows little role in colony formation.
- uvr^- confers great sensitivity to killing (colony formation).
- The effect of uvr^- survival sensitivity to UV could increase, but not decrease, the uvr^- strains' filament ratios.
- uvr^- limits the "strength" of the filament distribution relative to uvr^+ (which follows from uvr^- sensitivity to UV killing).

Current research in the induced repair system has led to the hypothesis that mutagenic inducers (largely producing pyrimidine dimers) leave post-replication gaps in DNA. These gaps signal the activation of the induced repair (rec A) products (i.e., Protein X). The uvr^- genotype is especially sensitive to these inducing influences presumably because, lacking excision repair, most induced dimers become gaps. In contrast, uvr^+ has, at least, the mechanism to repair some of these dimers before they reach the replication fork. The uvr gene acts on the DNA at the level of the initial induction by the mutagenic influence. The lon gene product is thought to be a secondary effect of the products of the induced system (e.g., Protein X). Induced products act on the lon gene product resulting in a lack of septation. The lon^+ gene is thought to lack this property of extreme sensitivity to rec A products.

Hypothetically, then, uvr determines the amount of rec A products produced, and the consequent effect on lon (septation). The entire scheme is illustrated below:



Clearly, the experimental results conflict with this model of the mechanism of filamentation. Particularly, the expected maximum for the uvr$^-$ lon$^-$ strain is not evident--uvr$^-$lon$^+$ is the most inducible strain. Here, however, the post-incubation plating data are quite informative. While the uvr$^-$ lon$^+$ strain shows a 5% increase in inducibility over the uvr$^-$ lon$^+$ strain, the uvr$^-$ lon$^-$ shows a considerably lower survival ratio at this dose (see Tables A. and B.). Thus, part of this difference in induction may be accounted for by a greater degree of cell death before filamentation in the uvr$^-$ lon$^-$ strain--thus reducing the apparent induction ratio. (Note also that the uvr$^-$ lon$^-$ strain shows declining slope at a lower dose than the uvr$^-$ lon$^+$ strain.) The fact that the lon$^+$ strains (particularly the uvr$^+$ lon$^+$ strain) are induced at all is somewhat surprising since this strain is considered insensitive to the induced products. The apparent difference in inducibility of these strains may only reflect the extent rather than the amount of septation failure.

Another point of interest lies in the similarity of the uvr^+ lon^- and uvr^- lon^+ curves which seems to undermine the importance of dimer formation in filamentation.

These observations challenge several aspects of the traditional model for induction of filamentation. Further research is needed to establish the exact link between induction and the failure of the septation mechanism.

Acknowledgment: This work could not have been completed without the interest, guidance, and assistance of Dr. D. J. Fluke and Dr. E. C. Pollard.

ACTION SPECTRUM FOR THE INDUCTION OF K12λ

There are four commonly used indicators of the induction of a prophage: the killing of the cells; the formation of infectious centers shortly after the administering of the inducing agent; the formation of free phage after time has been allowed for release of the induced phage; and the observation of the drop in turbidity of the cells due to the lysing of the cell wall by the phage endolysin. All have some qualifying disadvantages. Cell killing is not wholly correlated with phage induction: cells which will not form a colony can nevertheless be induced to produce phage. The formation of infectious centers shows a decline during the first 35 minutes or so before the actual phage multiplication begins. Free phage is a clear indicator, but there is a change in burst size with inducing dose which makes the yield hard to quantify. Drop in turbidity is not very significant until a large fraction of the cells have been induced because the uninduced cells continue to grow and maintain the turbidity.

Because each one of these methods has some question it was decided to observe three of them on the same culture and, in addition, to increase the sensitivity of the turbidity method. Accordingly we observed cell killing, by measuring the loss of colony forming ability, the formation of infectious centers as observed by plating very soon after induction,and we used cells which had been grown on lactose and so contained about 5% β-galactoidase, to give an indication of the destruction of the cell wall, which permits cells to give a color with the indicator compound ONPG. The uninduced cells dont give such color, so if they multiply they introduce only a secondary correction. We found that the dose response curves for these three methods dont give identical findings, but the major difference seems to be in the observation of the action of

the endolysin, and this is not greatly different from the other two.

In an action spectrum the dosimetry is crucial. We prefer to observe the action in the medium in which the cells are able to grow and if this is done there is, of necessity, some absorption. In addition, the cells themselves scatter light. Our technique was to irradiate with the double monochromator, which delivers a vertical beam, with the sample in a silica dish placed in the beam and above a photovoltaic cell read with a Keithley electrometer. Readings of the incident intensity are taken with distilled water in the dish and correction made for the reflection at the liquid surface. The sample is then introduced into the dish and during exposure the amount of light as recorded by the photovoltaic cell is measured and recorded. At the conclusion and just before exposure the amount of light recorded without the ~~cell~~ dish is measured. With this information the average exposure can be calculated in terms of the meter readings. To calibrate the meter two observations are taken. The first is a measure of the light intensity at the same location as the photovoltaic cell, using a thermopile and sensitive galvanometer, with provision for calibration at will against a standard lamp.The second is a field distribution over the area of the beam, from which, by a computer program which is standardized, the lateral average intensity can be found.

Plating for colony forming ability.

While there is no special problem about plating to determine cell survival, care has to be taken to secure as uniform plating as possible. Dilution is by menas of selectapette pipets set at 0.6ml into 5.4ml C minimal salts dilution blanks, prepared with an oxford pipetter. These survive autoclaving reasonably well, though it might be an improvement to use sterile tubes and a sterile pipetter with sterile medium to secure uniformity of dilution. 0.6ml samples are taken from the irradiation dish into 5.4ml of dilution blank, kept cold

in the refrigerator until time to dilute and plate. Dilution is done from the weakest to the strongest, using the same pipet for time saving. Dilution of 11 samples to 10^6 fold dilution can be done in a matter of minutes. For plating, 0.5ml of the 10^6 dilution (or for higher doses 10^5 dilution) is pipetted on to a bare dish (it is wise to have these prelabeled) and about 12-15ml warm agar poured over, with gentle swirling after. This is done with the "tip bottle". Care in wrist action will prevent agar all over the floor. The agar sets more rapidly on the stone bench and all plating for this experiment has been done there. It is essential to have the agar cool enough when it is poured so that hand holding of the tip bottle is not a feat of bravado. To ensure this, continual filling of the tip bottle, with care that sterility is maintained, from a stock bottle in the warm oven is wise. About 15 dishes to a pouring is about all that should be attempted before a refill.

When set hard the dishes are placed, in stacks of 2, in the incubator, set at about 37. Counting is feasible after 18 hours and better after 24. It is readily done at 48, but the plates get a bit solidly grown by 72, though they should not be discarded. 3 plates to a single dilution is reasonable. 4 would not be excessive.

Plating for infectious centers.

This plating is done as soon as is reasonable after giving the UV. The sample of 0.6 ml into 5.4ml salty broth is taken at the same time as the sample for colony forming ability. Trivial precautions to be sure the samples are correctly taken are very wise. The samples so taken are kept cold and dilution to 10^5 in salty broth (refrigerator cold) is done right away. 5ml blanks of soft warm agar (44^oC) are prepared, sterilized in the autoclave and brought into use in 2 stages of temperature control. The sterile blanks are put, while hot, into

nearly boiling water and withdrawn, 6 tubes at a time into the 44^0C bath. Two are selected, felt for temperature after a short time, and 2 drops of Q1 cells derived from an overnight slant by adding 5ml of salty broth and thoroughly mixing, added. 0.5ml of the phage dilution is thenpromptly added, a short vortex given and the whole poured on to the surface of a hard agar containing.dish. These agar dishes must be prepared the day before. These dishes are also lighly swirled, the lid replaced and an indicator cap of some kind placed on the last dish . Plated dishes can not be told from those awaiting plating and it is wise to have some system to guarantee against double plating. The plates are put into the incubator reasonably soon after hardening. Plaques dont develop at 23^oC or so. At low inducing doses 0.7ml infectious centers were plated and, on occasion, more plates used.

Upon looking at the plates after about 16 hours incubation time, those which have had low or no doses will be found to have two classes of plaque. One is rather small with a hard white small colony in the center. These are the free phage plaques. The time available for the free phage to attach is so long that essentially all the freephage is attached to bacteria. They take time to come off and find the non-immune indicator strain. There they make a plaque, but at the same time the immune cells make a very successful colony in therich lysate. In contrast, the induced cells produce a burst of phage which are unattached and immediately begin to operate on the indicator strain. They then produce a larger plaque with the normal cloudy center due to thecells which have become lysogens developing. This is not the hard white center but is more diffuse. With care it is readily possible to distinguish the two and count only the infectious centers.

Observation of cell lysis.

E.coli K12λ will readily grow on lactose casamino acid medium. Cells grown in glucose or broth, however, take some time to make the transition to lactose. Accordingly a culture of K12λ is first grown to an O.D. of about 0.3 on glucose and then transferred to lactose medium overnight. This will give a thick culture which should be transferred to fresh medium in the morning. The lactose culture can be refrigerated and used as stock for up to a week, or can be renewed from time to time. It was our practice to return to a slant once a week and go through the process of transfer from glucose to lactose.

The lactose grown culture is used in the primary irradiation process. 3 ml samples are taken into sterile flasks held in ice and these are accumulated until the whole irradiation process is complete. The set of flasks are then put into the shaking water bath for a time of between 120 and 150 minutes. A series of test tubes containing 5ml of Cminimal salts and 0.5ml of ONPG at 1.4 mg per ml is set in a water bath at 34°C. At the end of the incubation time two 0.5ml samples of incubated cells are added to two of the tubes with salts and ONPG. The cells which have received none, or low doses are allowed to act on the ONPG for20 minutes and those which have been more fully induced for only 5 minutes. This prevents saturation of the color substrate. The reaction is stopped by adding 2ml of 0.5M sodium carbonate to raise the pH. Some increase in color is seen when this is done. A Cornwall pipette is convenient for this part.

The contents of the tubes are then centrifuged to pellet the cells and the supernatant read at 425nm in the B&L spectrophotometer. Previous work suggests that the amount of color accurately measures the amount of enzyme activity. OD versus enzyme amount for a fixed time gives a

INSERT JUST UNDER "RESULTS"

In order to make use of the data obtained as described above some normalization is necessary. The most direct datum is obtained from the comparison between the titer observed for infectious centers and the titer of colony forming ability. When the background phage count for no dose has been deducted, the infectious centers yield at once the number of induced cells per ml. This can be divided by the number of CFA recorded cells to give a percent induced cells. In general this has shown a maximum at about 40%. This maximum is then used together with the maximum for the amount of enzyme activity to normalize the data pertaining to lysis. There is no problem here. In order to bring the cell killing data into the same scheme the procedure adopted, which can be seen on Figure 3.3, was to draw the best line through the data points and then take any drop from the zero dose titer as the cells which have been killed. The assumption is made that for low doses the killing is due to induction. This number of cells killed is then expressed as a percent of the zero dose titer, derived from the best line through the data, and a table prepared. To normalize, it has been assumed that the number of cells killed at the dose which gave a maximum for the infectious centers assay is the number killed by induction at that dose. Further killing is assumed to be due to damage to the cell other than induction. This is not very satisfactory, but no better scheme has presented itself. The percent cells killed is then normalized just as for the observation of lysis. As explained later this procedure may give a line which is deviant from the others, but the initial kinetics should not be misleading. Probably the plotting of percent induced in this way is not very far away from that found for infectious centers and a systematic difference could be significant.

proportionality. The amount of color read is also proportional to the time so long as saturation is not approached.

The experiment thus gives a series of OD readings for a series of doses. The short enzyme reaction times are prorated to the long by the needed ratio (4 in the above case), and there results a reading of yield of induction versus dose.

RESULTS

A characteristic set of dose response curves obtained in this way is shown in Figure 3.1. This is the result of one days experiment with exposure at 289nm. The dose figures have not been corrected for lateral distribution and are about 60% too high. The characteristic behavior seen is that of a slightly sigmoidal increase in the percent of cells induced. In this experiment the observations for the infectious centers and for the lysis of the cells go together very well, while there is some separation for the case of cell killing. Part of this separation may be due to the method of normalization, which is to take the maximum found in the plating for infectious centers and make the other two methods conform to it. This is quite sensible for the observation of lysis, where there is clearly a maximum, but it is rather arbitrary for cell killing, which proceeds to very small surviving fractions as the dose is increased. It is reasonable to conclude that the lower slope at low doses for cell killing is real, but not that the whole line is lower.

In Figure 3.2 we show data taken at 265nm for two physiologically different cultures. The first case is that of normally grown cells in lactose casac medium, and the second that of cells grown in lactose and nutrient broth. What is of interest is that in the latter case the lysing of the cells occurs at definitely lower doses than either the

killing or the formation of infectious centers, while in the casac [illegible] case the separation is not so large and the formation of infectious centers occurs al lower doses. These differences due to physiological state were pointed out many years ago by Lwoff: it will be of interest to see if they can be correlated with the number of genomes per cell and whether they are different at different wavelengths.

In order to plot an action spectrum for each of these indicators of induction it is really necessary to derive an analytical function for the dose/response curves. This was done by Franklin in his early work, but our findings differ from his in two significant ways. The first is that the induction, as measured by infectious centers, or by lysis, does not rise sharply at the low to zero dose region, but starts more sigmoidally. The second is that our observations of cell killing, as exemplified by Figure 3.3, show a shoulder and do not fall exponentially from the start. Plotting our data on a log yield/log dose basis does not reveal any simple dose dependence as is found for leucine revertants. We have, therefore, in what we hope will be a temporary solution, read off the 50% maximum induction dose for each process and each wavelength and taken the reciprocal of this as a figure of merit for induction. The resulting action spectrum is shown in Figure 3.4. Here are shown $1/D_{50}$ values for the three processes we have observed, plus data values given to us by Dr. W.R.Guild for the absorption of pure calf thymus DNA, plus the points obtained by Franklin, replotted to fit our graph.It can be seen that on the long wavelength side, not reported by Franklin, the fit to the DNA absorption is excellent. The range of 10^4 covered also shows that the short wave contamination of longer wavelengths has been well controlled. On the other hand, the maximum for induction in both our work and that of Franklin lies at longer wavelenths than the DNA absorbance and is also sharper. This suggests that it may be worth while to pay more attention to the shorter wavelength lines in future work.

It is possible that the lesser response at shorter wavelengths may be due to the destruction of dimers in addition to their creation. This seems to be unlikely, both on the grounds that quite small doses are being used, and also as can be seen from Figure 3.5, where data taken in the same manner on the killing of strain B_{s-1} (lex^-, uvr^-) are plotted. Here the fit to the DNA absorbance is much better. Moreover, in this series of experiments each survival curve plotted at each wavelength was coupled with a fully photoreactivated survival curve. The fraction photoreactivable varied between 75 and 79% for all the wavelengths used. This is good presumptive evidence that the killing of B_{s-1} is due to unremoved dimers, since the photoreacivating enzyme predominantly acts only on dimers. Thus we feel that the deviation from DNA absorbance at the short wave end may have further significance.

It is our intention to observe the action spectrum for one other aspect of induced repair in strain K12 to see whether this drop at short wavelengths is maintained for the derepression of <u>recA</u> without necessarily having anything to do with the induction of λ.

WAM-D4

INDUCTION OF INDUCED REPAIR BY UV GIVEN TO CELLS IN THE FROZEN STATE.

Ernest C.Pollard, Mildred L. Rader and Donald J. Fluke, Department of Biochemistry and Biophysics, The Pennsylvania State University, University Park, PA and Zoology Department, Duke University, Durham, N.C.

Observation of the induction of inhibition of post radiation DNA degradation and of induced radioresistance shows that UV given to E.coli cells in the frozen state acts as an excellent inducer of the induced repair (S.O.S.) system. The induction is considerably less photoreactivable than is the case when UV is given to the cells in the liquid state. Cells are prepared for freezing by filtration of Millipore filters and then rapidly frozen in a metal dish in contact with Dry Ice and methanol. Kept in this condition the recovery of viable cells can reach 50%. The viability of cells after exposure to UV in the frozen state is less than that of cells in the liquid state and the photoreactivability is less. Mutagenesis by UV given to frozen cells, as tested by observation of leucine revertants in strain WU 3610-89 and tryptophan revertants in strain WP2s tif 1, N.F. is comparable with that produced in the liquid state but the revertants are not 95% photoreactivable. Cells irradiated in the frozen state are believed to contain a high proportion of DNA-protein crosslinks. This work suggests that such damage elicits the induced system effectively and that a part of its mutagenic action may be due to that fact. (Supported by E.R.D.A. Contract (11-1) 2362).

(WAM-D) UV DAMAGE AND REPAIR

WAM-D1
WAVELENGTH DEPENDENCE OF ULTRAVIOLET MUTAGENESIS IN RELATION TO INDUCED REPAIR. By Donald J. Fluke and Ernest C. Pollard, Department of Zoology, Duke University, Durham, N.C. 27706

Reversion to leucine independence has been examined in uvr⁻, tyr⁻, leu⁻ strain of E. coli B/r, at ten wavelengths from 234 nm to 313 nm. The ultraviolet mutagenic exposures, employing a double water-prism monochromator, have routinely tested the full mutagenic expression range, from background to the high dose limit for recovery of revertants at constant plating aliquot. After mutagenic exposure cells have been grown on limited leucine to a microcolonial stage, among which revertants are counted as macrocolonies. Colonial survival has been simultaneously tested on fully supplemented agar medium. At all wavelengths the mutagenic expression, as revertants per survivor (R/S), follows a dose-squared dependence at lower ranges of UV exposure. At the mid wavelengths, represented by 254 and 265 nm, two further phases of mutagenic expression have already been reported: a linear or less-than-linear dependence upon UV exposures in the mid-range, and a steep increase in R/S at the high range of UV exposure. At higher wavelengths, 300 nm and above, the lesser dependence at the exposure mid-range is absent, with dose-squared dependence out to high exposures. At the low wavelength, 234 nm, R/S declines at highest exposures. Induction of error prone repair and production of pre-mutational damage are interpreted to show somewhat different wavelength dependencies. (Work supported by ERDA/DOE Contract No. EY-76-S-05-3631, and assisted by John K. Douglass).

The work reported by abstract entitled "Wavelength dependence of ultraviolet mutagenesis in relation to induced repair" was presented at the Sixth Annual Meeting of the American Society for Photobiology, Burlington, Vt., in June.

Fig. 1 shows the method we developed for correcting background mutagenesis. The background mutant yield does not fall off directly with dilution as a model for inactivation, but is not constant either. The exact form of this correction is critical only at high doses where the mutant yield curve has again declined toward background levels.

Fig. 2 summarizes our rationale for distinguishing separate values of induction and pre-mutational damage sensitivities in any one experiment.

Figs. 3-7 show results of individual experiments at two extremes and one middle wavelength. Two are repeats at the same wavelenth, to show run to run variability. Each set of data is plotted three ways: as a yield curve corrected only for background, as a revertant to survivor ratio dose-action curve, and as our inferred induction curve (R/SD). The extent of dose-squared mutagenesis is seen in these results. At low wavelength we see a downtrend at high dose, and at mid and long wavelength an uptrend. The mutagenesis dose-action appears to involve several distinguishable phases.

The most constant feature we find in these experiments (five from among some fifteen) is the maximum yield value, 3000-4000 revertants/ml.

In Figs. 7 & 8 the pre-mutational and induction sensitivities are shown as action spectra, with a DNA absorption spectrum superimposed for comparison. While not inconsistent with DNA as chromophore these results are clearly too noisy for a tight indication of UV absorption via DNA. Some of the noise is experiment to experiment, but is amplified by separating two parameters within the data. It is of interest that the induction sensitivity is some 10^5 larger than the premutational damage sensitivity.

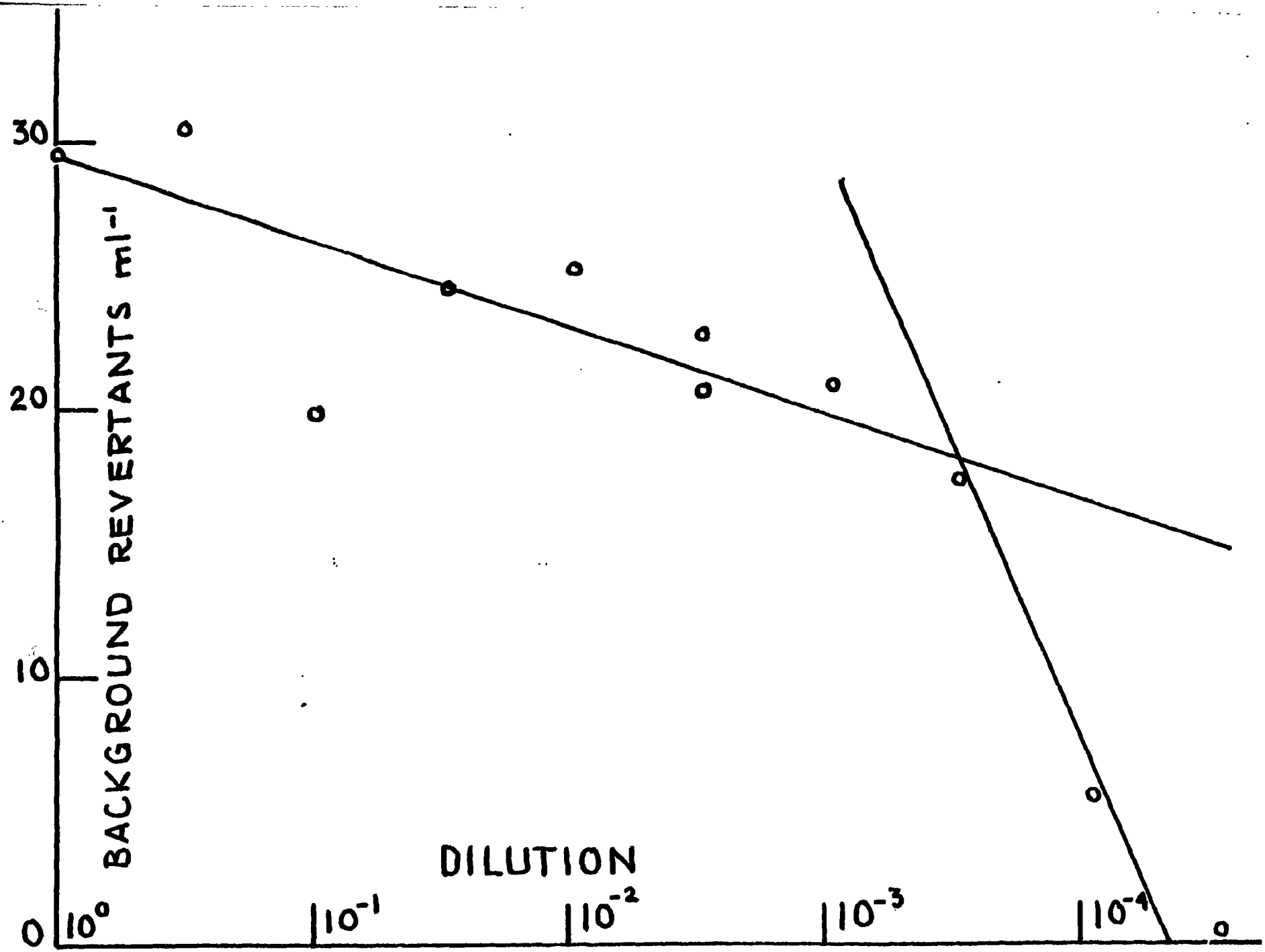
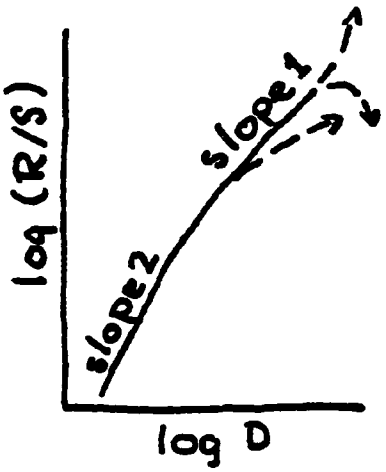

30
20
10
0
BACKGROUND REVERTANTS ml⁻¹
DILUTION
10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴

Fig. 1

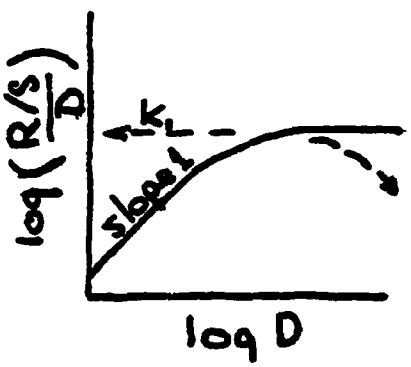


$$R/S = k_1 D (1 - e^{-k_2 D})$$

or

$$k_1 D (e^{-k_3 D} - e^{-k_2 D})$$

$$(R/S)_{D\,small} \longrightarrow k_1 k_2 D^2$$



$$\frac{R/S}{D} = k_1 (1 - e^{-k_2 D})$$

$$k_1 = \left(\frac{R/S}{D}\right)_{D\ large,\ k_3 = 0}$$

$$\left(\frac{R/S}{D^2}\right)_{D\,small} = k_1 k_2$$

$$\left(\frac{R/S}{D}\right)_{D small} \Big/ \left(\frac{R/S}{D}\right)_{D large} = 1 - e^{-k_2 D_{sm'l}}$$

(yields k_2)

$$\left(\frac{R/S}{D^2}\right)_{D small} \; ; \quad / k_2 = k_1 \; ; \quad / k_1 = k_2$$

Fig. 2

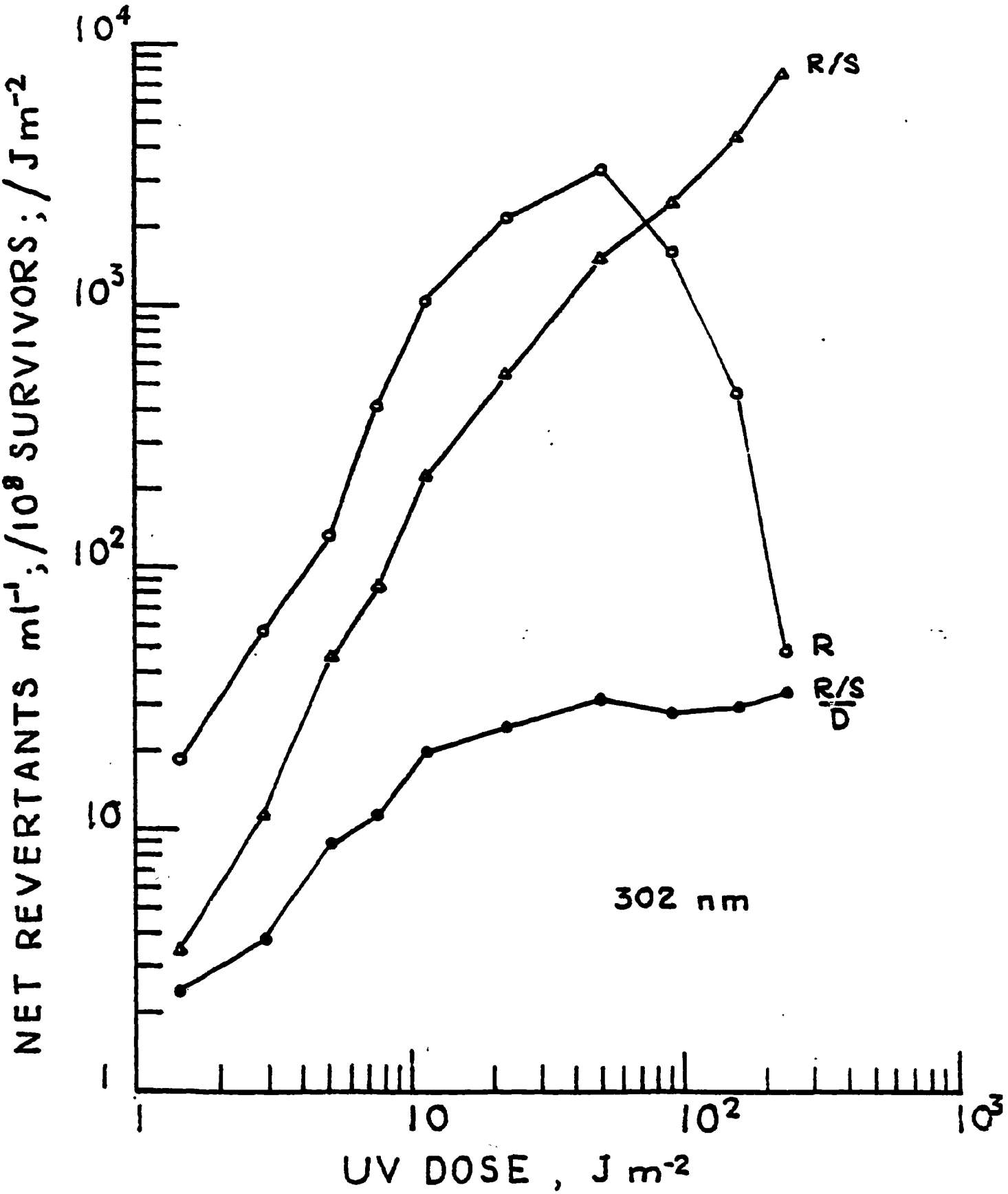
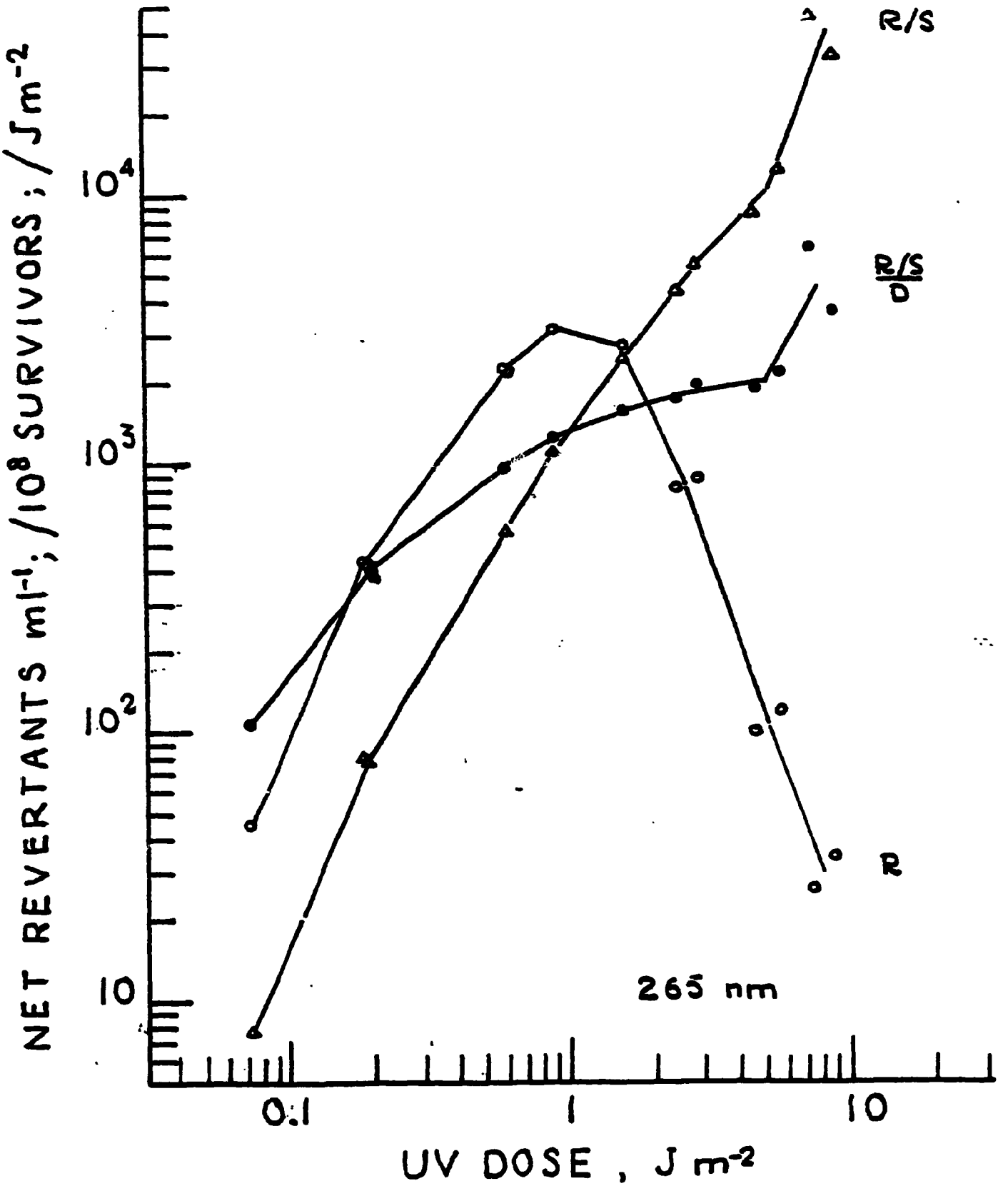

NET REVERTANTS ml⁻¹ ; /10⁸ SURVIVORS ; / J m⁻²
10⁴
10³
10²
10
1
R/S
R
R/S / D
302 nm
1
10
10²
10³
UV DOSE , J m⁻²

Fig. 3


NET REVERTANTS ml⁻¹; /10⁸ SURVIVORS ; / J m⁻²
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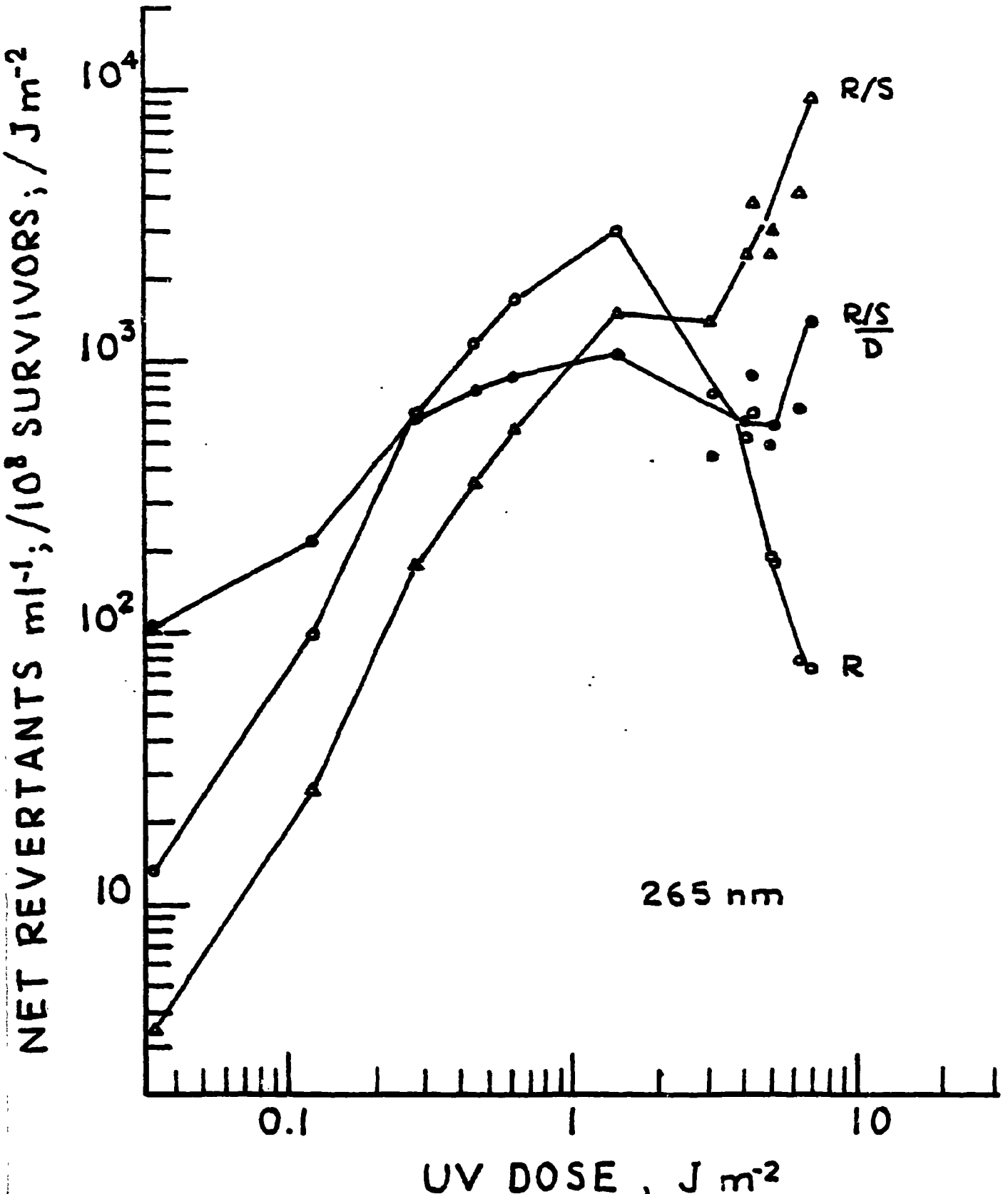
10³
10²
10
R/S
R/S / D
R
265 nm
0.1
1
10
UV DOSE , J m⁻²

Fig. 4


NET REVERTANTS ml⁻¹; /10⁸ SURVIVORS; / J m⁻²
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10³
10²
10
R/S
R/S/D
R
265 nm
0.1
1
10
UV DOSE , J m⁻²

Fig. 5

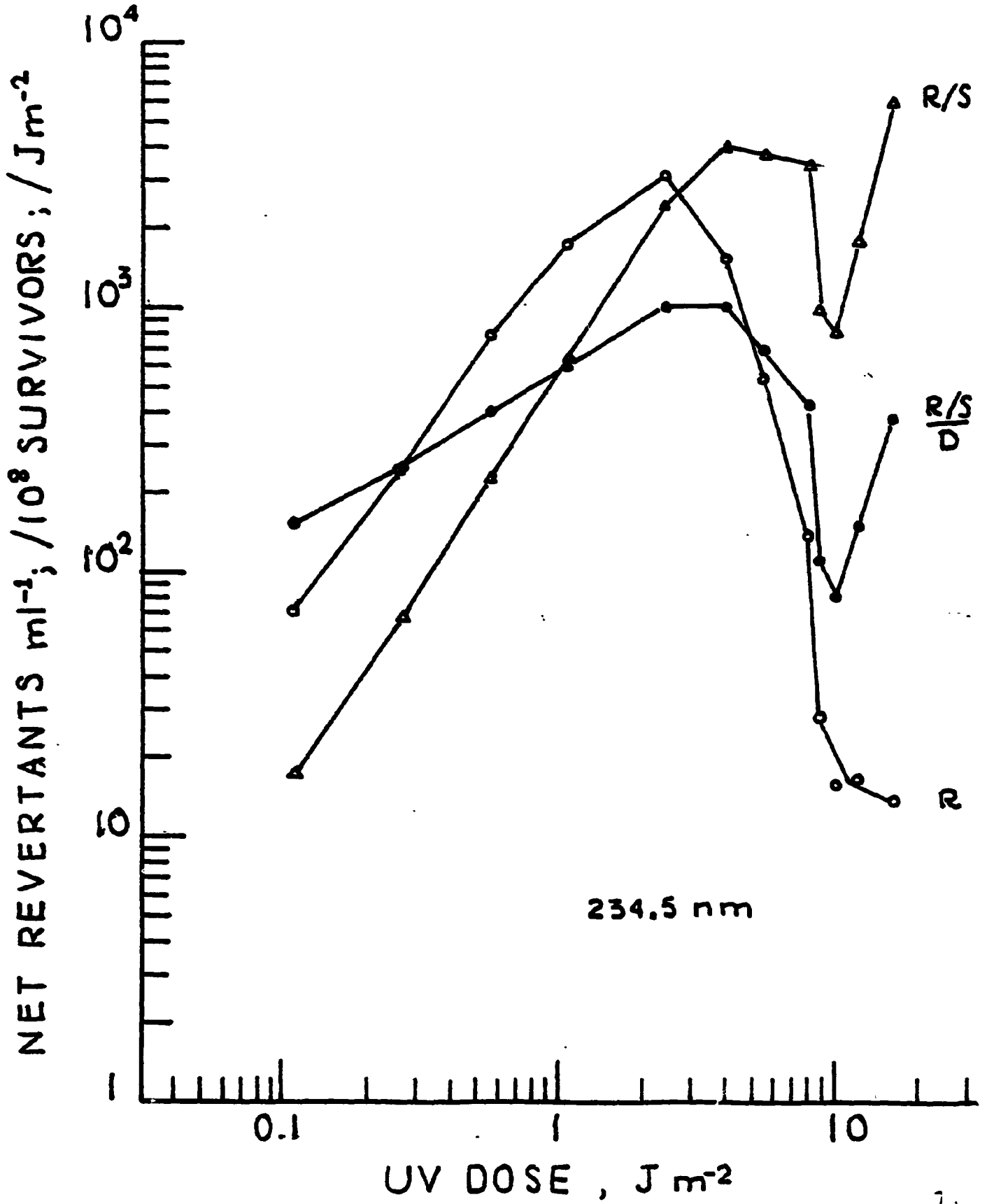

NET REVERTANTS ml⁻¹; /10⁸ SURVIVORS ; / Jm⁻²
10⁴
10³
10²
10
1
0.1
1
10
UV DOSE , J m⁻²
R/S
R/S / D
R
234.5 nm

fig. 6

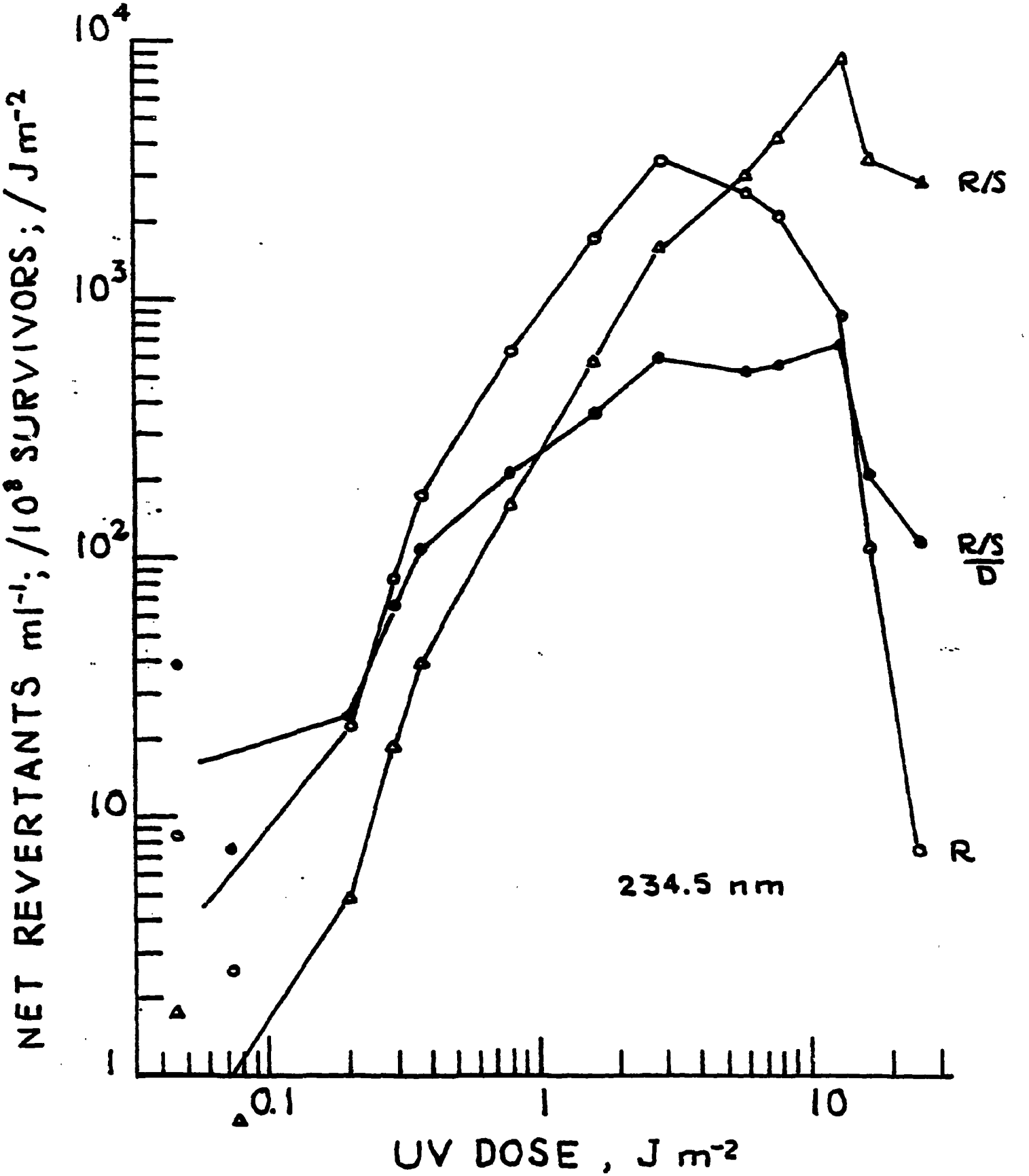

NET REVERTANTS ml⁻¹; /10⁸ SURVIVORS; / J m⁻²
10⁴
10³
10²
10
1
0.1
1
10
UV DOSE , J m⁻²
R/S
R/S/D
R
234.5 nm

Fig. 7

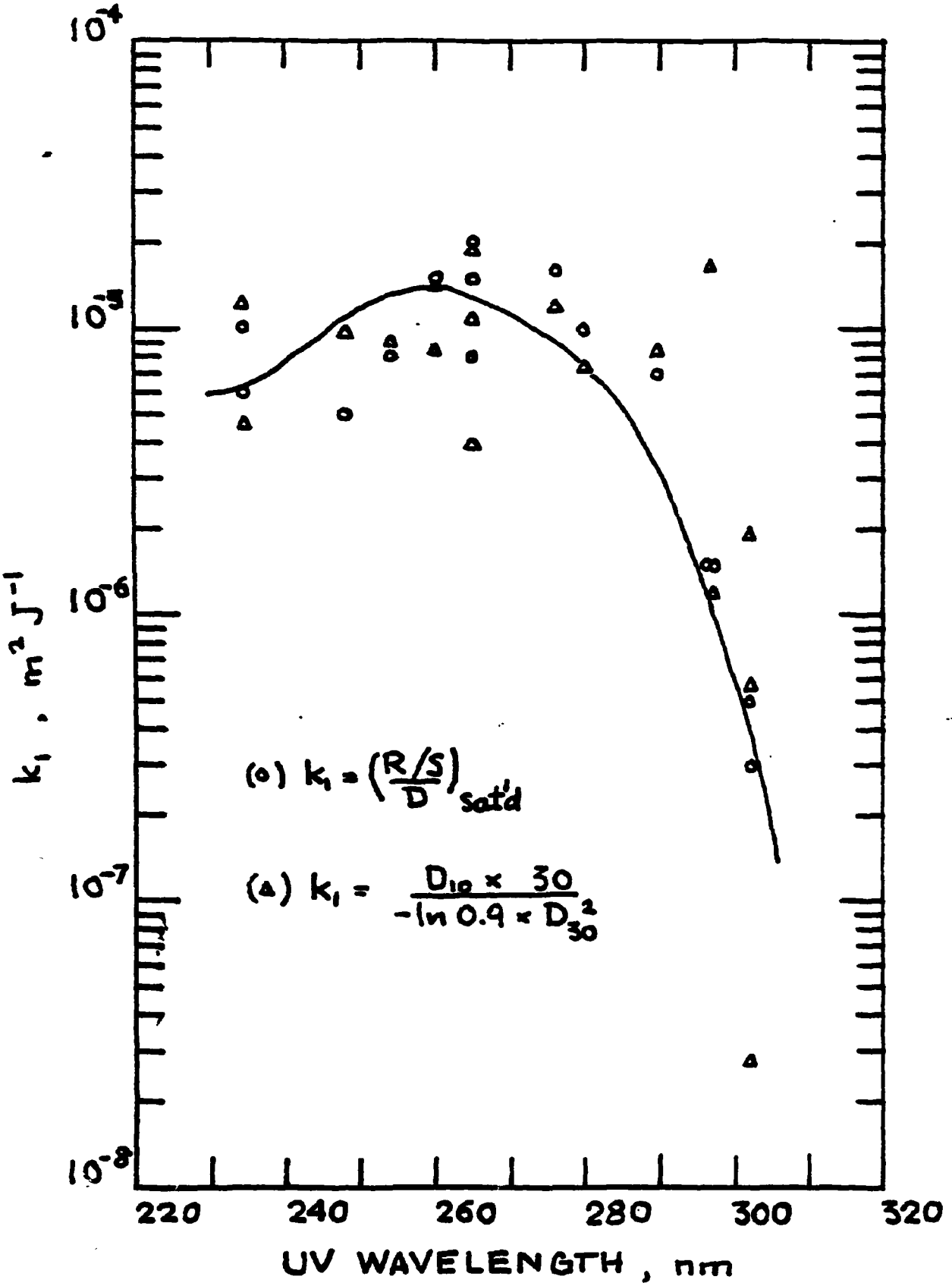

10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
k_1 , $m^2 J^{-1}$
(o) $k_1 = \left(\frac{R/S}{D}\right)_{sat'd}$
(Δ) $k_1 = \frac{D_{10} \times 30}{-\ln 0.9 \times D_{30}^2}$
220
240
260
280
300
320
UV WAVELENGTH , nm

Fig 8

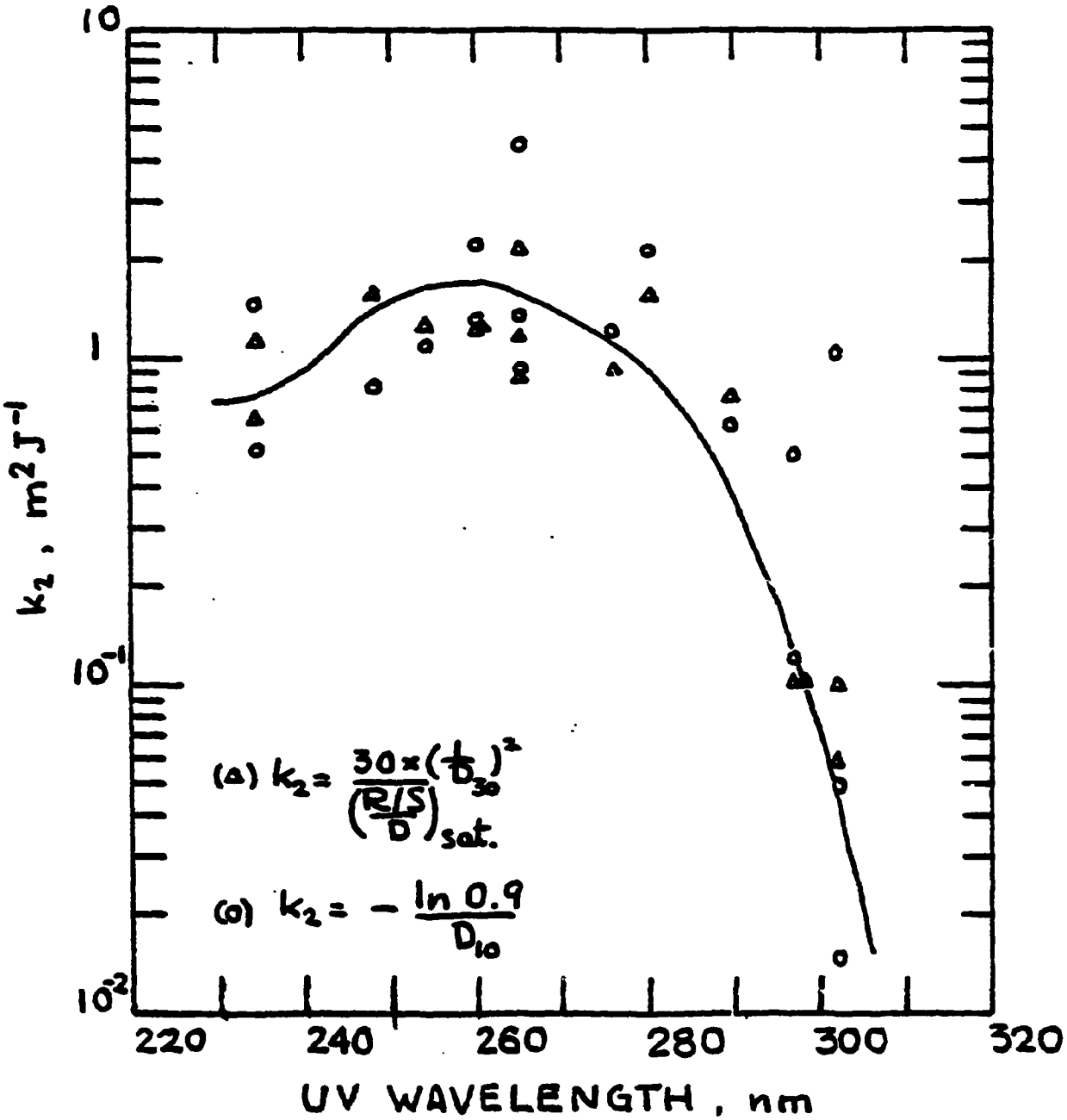

10
1
10^{-1}
10^{-2}
k_2, $m^2 J^{-1}$
220
240
260
280
300
320
UV WAVELENGTH, nm
(a) $k_2 = \frac{30 \times (\frac{1}{D_{30}})^2}{(\frac{R/S}{D})_{sat.}}$
(b) $k_2 = -\frac{\ln 0.9}{D_{10}}$

Fig 9

(Ge—9) *Repair of X-ray Damage in Phage λ by UV Exposure of E. coli Host Cells.* D. J. FLICKE AND E. C. POLLARD, Department of Zoology, Duke University, Durham, North Carolina 27706.

Weigle-reactivation, the repair of UV-damaged phage in UV-induced bacterial host cells, appears also to have some effect in enhancement of plaque survival of x-ray-damaged phage λ. Inductates of phage λ were irradiated by 50-kvp x-rays both in 5× nutrient broth (890 krad) and buffer (200 krad), to 10^{-4} survival. *E. coli* AB1157 grown in a minimal salts + maltose + casamino acids medium was transferred into the buffer medium appropriate for phage adsorption (0.01 $MgSO_4$ in 0.01 *M* Tris-HCl) and were UV-irradiated by a 4-watt germicidal lamp, for 0, 2.5, 5, and 7.5 Jm^{-2} at 254 nm. Upon adsorption of the x-ray phage λ to these cells, plated for infective centers after 15' at 37°C, reactivation factors of 1.4 to 2× that for the same x-ray-phage on non-UV cells was observed. On the same UV-host cells UV-damaged phage λ at 10^{-3} survival showed reactivation factors up to 15×, increasing with UV exposure of the cells. The reactivation of x-ray damage is much less, and saturates at lower UV exposure of the host, but does appear to be established in these experiments. (Work supported by DOE, contract No. EY-76-S-05-3631.)

The work reported by abstract entitled "Repair of X-ray damage in phage λ by UV exposure of E. Coli host cells" (Radiat. Res. 74, 1576 (1978)) was presented at the 26th Annual Meeting of the Radiatiou Research Society, in Toronto (May 1978). Three figures are included from the presentation.

Fig. 1 shows the survival ratio of UV-inactivated phage λ in E. coli K-12 host cells which have been induced by various doses of 254-nm UV. The 10-fold rise in survival is the well-known Weigle-reactiviation. A consistent feature of such study of UV-damaged phage λ in our hands is the considerable drop in phage survival on forming complexes with the AB1157 host in adsorption liquid medium. The same holds for making complexes with E. coli Q_1, the seed host.

Fig. 2 shows a less but still appreciable W-reactivation of X-ray exposed phage λ, both for protected irradiation in 5X concentrated nutrient broth, and for less protected phage in the $MgSO_4$-Tris-H Cl adsorption medium. The difference in dose to the phage for the same survival in the two media is apparent. For X-ray damaged phage we find an increase in survival on forming complexes with the E. coli K-12 strain in liquid medium. The increase does not occur for unirradiated phage diluted to the same level.

Fig. 3 shows an experiment directly comparing reactivation of UV and X-ray damage in the phage, in the same irradiated host cell preparation.

fig. 1

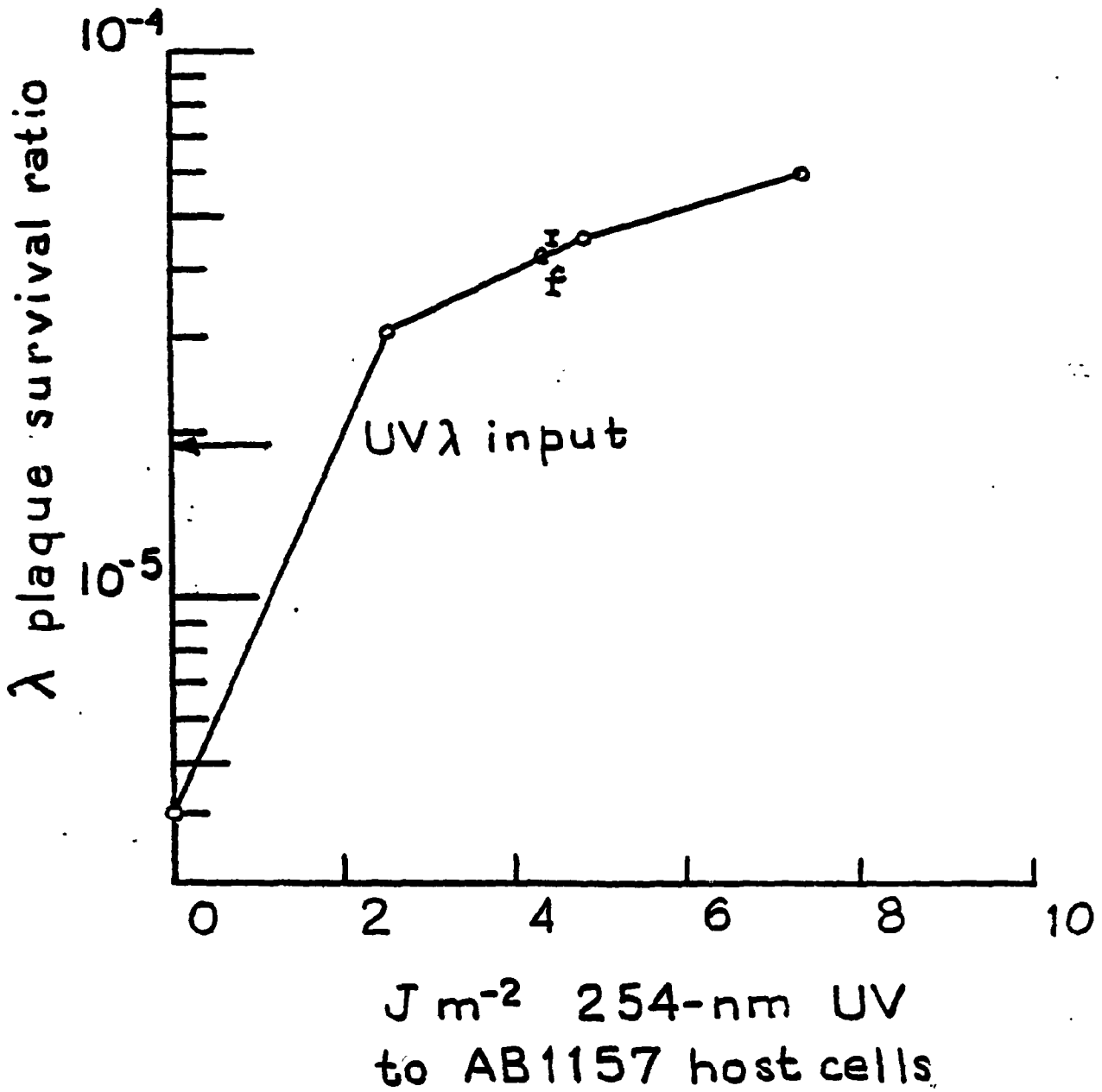
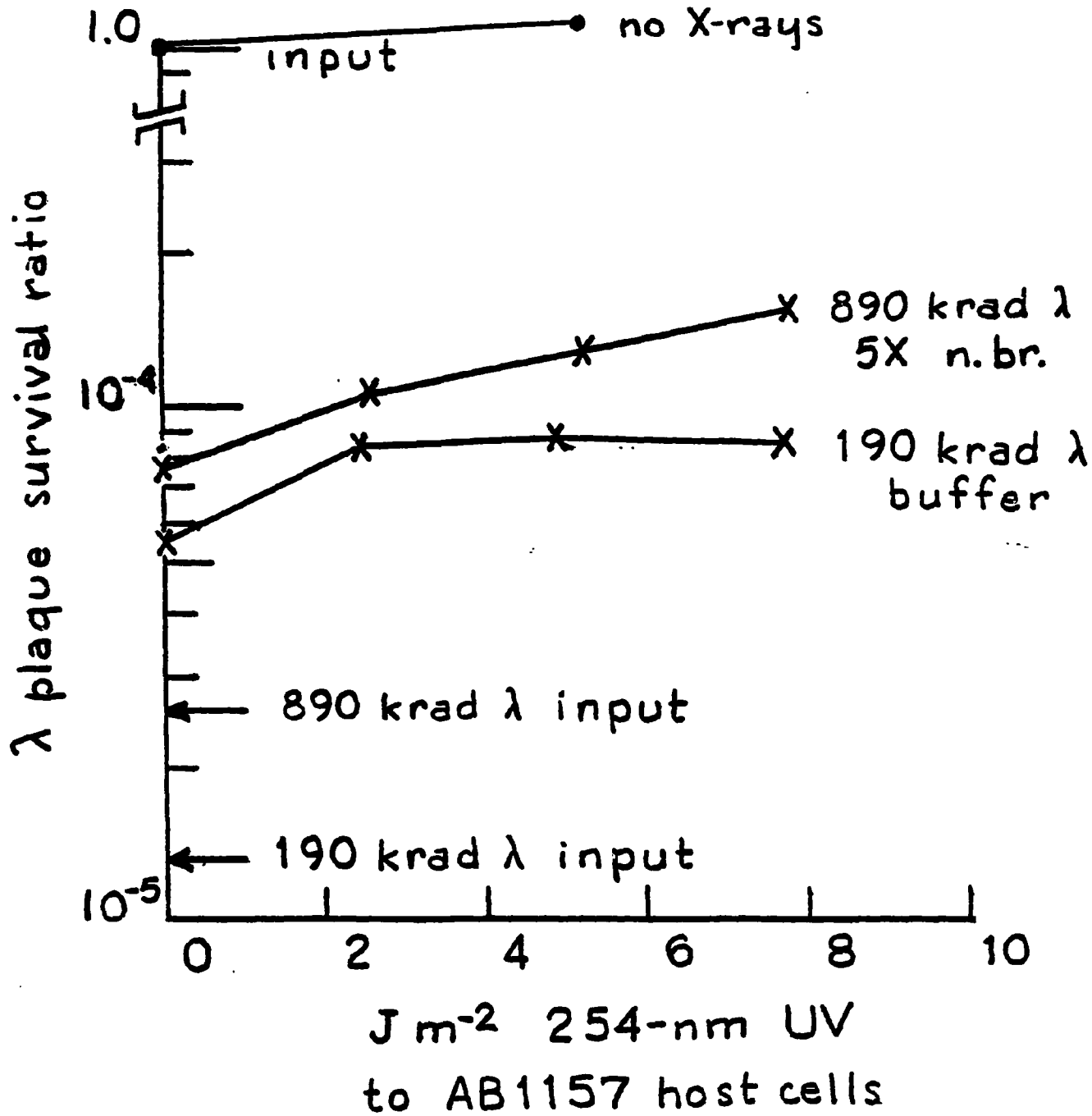

λ plaque survival ratio
10⁻⁴
10⁻⁵
UVλ input
0
2
4
6
8
10
J m⁻² 254-nm UV
to AB1157 host cells

Fig. 2


1.0
input
no X-rays
890 krad λ
5X n.br.
190 krad λ
buffer
λ plaque survival ratio
10^{-4}
890 krad λ input
190 krad λ input
10^{-5}
0
2
4
6
8
10
J m^{-2} 254-nm UV
to AB1157 host cells

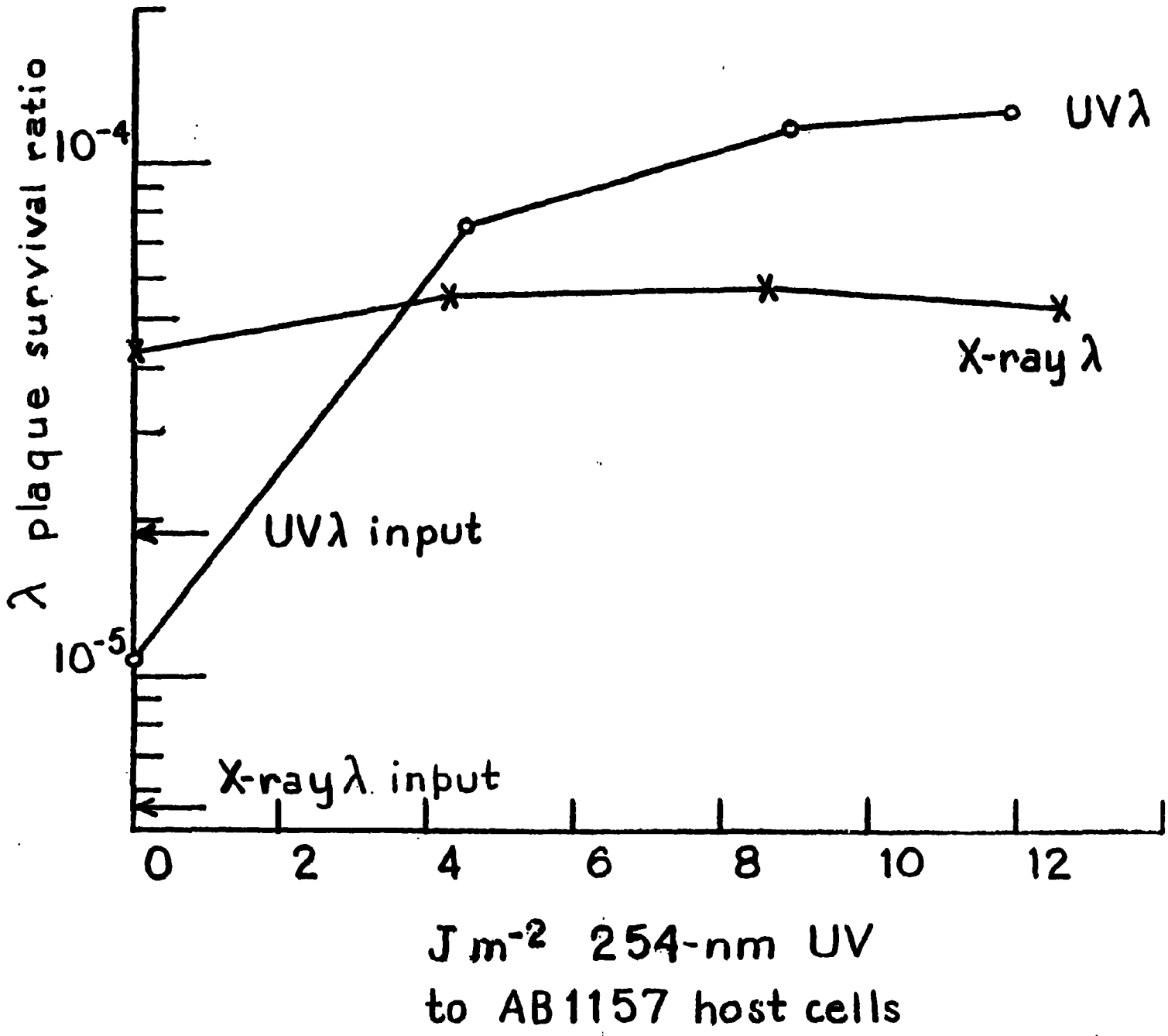

λ plaque survival ratio
10^{-4}
10^{-5}
UVλ
X-ray λ
UVλ input
X-ray λ input
0
2
4
6
8
10
12
J m^{-2} 254-nm UV
to AB1157 host cells

Fig. 3