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RADIATION DAMAGE AND REPAIR IN

ELLS AND CELL COMPONENTS

78 - 1979 Report

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RADIATION DAMAGE AND REPAIR IN CELLS AND CELL COMPONENTS

Progress Report: Second New-Contract Year

INTRODUCTION

Personnel

The group involved in this project include D.J. Fluke, and E.C. Pollard, Co-principal Investigators, Deno Kazanis, Hargitt Research Fellow: Brian Robinson, Richard Page and Anne Coleman, undergraduate research students working on special projects.

Major Thrusts

While the central theme is the understanding of the nature of the action of radiation, both UV and ionizing on cells and cell components, special work of the past year has concentrated on induced repair in a number of strains of *Escherichia coli*. A summary of the present state of knowledge of induced repair is included by way of introduction. The situation still demands work, as our summary indicates. (APPENDIX 1)

Five lines of work have been followed as the main effort: the question of coordinate induction of a number of phenomena associated with induced repair; the study of ultraviolet mutagenesis and its relation to induced repair; the study of the induction of phage λ in λ lysogens; the study of the dose effect and time course relations for the induction of the *recA* gene product - protein X in a number of strains and the observation of Weigle-reactivation of phage λ in different strains and for different insults to the phage. One objective is the determination of an action spectrum for the induction of various phenomena and for the induction of λ in particular.

Developments from this program

A research study opens up lines of work outside the main line proposed. In adding the quantitative dose-response studies on protein X, the *recA* gene product, Kazanis has found it necessary to look into a variety of mutant forms of *E. coli*,

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and a variety of inducing agents. These studies have suggested that a more incisive test of coordinate induction can be made with special mutants which show different induction behavior for the recA gene product and so should also show different induction behavior for other phenomena. This has been a very productive area and will form one solid publication.

It has also proved to be difficult to generate firm data on the induction of λ without some understanding of the factors at work in the various methods of assay for induction. For example, if the measure of induction is the formation of free phage after time has been allowed for burst, then allowance has to be made for the secondary fact that the induced cells are septum inhibited and so grow longer and may have a larger burst size. One is therefore simultaneously studying two phenomena, not a good way to study one. We are approaching the end of the background work on these problems and can shortly make a start. One added problem is the undoubted non-linearity of the induction of λ which makes the assigning of a parameter descriptive of induction difficult.

The work on ultraviolet mutagenesis is, in many ways, in the most satisfactory state. One publication which comes as a follow up to a report at the meeting of the American Society for Photobiology last year is a separation of the two factors in UV mutagenesis. There are some added factors at some wave-lengths, but the inductive and the premutagenic cross-section have been separated and their response to different wavelengths indicated.

The work on W-reactivation of damaged λ phage has led to the realization that both UV and ionizing radiation damage can be reactivated and that the latter produces reactivable damage when the irradiation is done in dry and vacuum conditions, i.e. anoxic. Present work is also showing an involvement of the uvr mutation in the extent of W-reactivation and suggesting that different treatments of the phage may elicit different responses in the UV treated cells. This moves thinking away from the broad concept that there is one induced "error-prone" repair system which is responsible for all the actions observed.

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We have also come to realize that the phenomenon of induced repair may have a profound effect on observation at low doses and their relation to the extrapolation down from higher doses. Put shortly, at very low doses the radiation insult can do either of two things in an all-or-nothing way. It can either induce the cell for induced repair, which now renders the cell resistant, or it can fail of such induction and, in consequence, inflict damage on a cell lacking one of the repair systems and hence abnormally sensitive. Thus sensitivity at very low doses might be much higher than expected. On the other hand such cells will not readily mutate, nor will they induce prophage. Thus if it were established that induced repair is clearly found in human cells, then predictions regarding low dose effects would be involved with considering two populations, one induced and the other not, and deductions from higher doses would have to take this dual population into effect. We have come to this realization only recently and are making plans for studies to show that it is a tenable hypothesis.

Several smaller findings form interesting material and will be reported. These will either be given in the body of the progress report or form appendices, the appendices being the draft forms of paper being submitted in most cases.

Specific Elements of the Program

I. Coordinate Induction in strains AB1157, AB1886 (uvr) and WU3610-89 (uvr).

In Appendix 2 we give the draft of a paper to be submitted either to the Journal of Molecular Biology or the Journal of Bacteriology. A word of explanation of the term "coordinate" is in order. All the pleiotropic responses of the induction of the recA gene product are considered to be a manifestation of the derepression of the gene. We consider that this needs proof. Accordingly we have chosen, as inducing agent, monochromatic UV light, which penetrates cells readily and has a definite action on the DNA. It can be quantitatively measured with the absolute dosimetry which is part of the technique available with the large Duke University UV water-prism monochromator. We then observe the dose-response relation

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for various phenomena associated with the derepression of the recA gene. These dose-response curves should be the same, or closely so. If they are, then we feel that they form part of coordinate induction. If they aren't then some other factor must be at work. The table below summarizes our findings on these three strains.

TABLE 1

For most of the phenomena there is a rough agreement with a relation fraction induced = $(1 - e^{-aD})$. Values of a are given and if they seem to be in the range conforming to coordinate induction a + is indicated. If the dose response is not of that form, this is stated and a - is shown. Dose-response behavior for various phenomena in the three strains designated.

Phenomenon	AB1157	AB1886	WU3610-89
Protein X	0.36	1.2	3.2
Induced inhibition of prd	0.28 +	1.7 +	3.6 +
Induced radioresistance	0.46 +	3.8 -	7.0 -
Septum inhibition	0.27 +	1.7 +	3.5 +
W-reactivation	0.40 +	Not done	Not done
UV mutagenesis	Not done	Very sigmoidal -	Revertants per survivor divided by dose give 3.6 +
Induction of λ	Sigmoidal -	Sigmoidal, but if divided by dose, 1.7	

The impression given by the table is not as convincing as the actual data, because it is apparent from the data that the form of the dose-response curves is very much the same for those marked +. In general the doses to induce protein X are somewhat higher than for the other phenomena, and this may reflect the time at which the assay for protein X was made. It may have been too short. In the "-" column are two phenomena. One is the induction of radioresistance. This

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usually follows the approximate $1 - e^{-aD}$ form, but it appears at roughly half the dose in many cases. We feel that this can be interpreted in terms of the end point in the observations, which is a colony, something that can develop from a single daughter, or even a granddaughter of a tetranucleate cell. It suggests to us that it is possible to induce only one of several genomes and that this is sufficient to give radioresistance.

The other negative phenomenon is the induction of λ, which clearly does <u>not</u> follow the $1 - e^{-aD}$ relation. In the case of AB1886 the data can be analyzed to suggest that one UV insult causes induction of protein X and that a second is needed to complete the induction of prophage. This does not work so simply for AB1157 where it looks more as though two $1 - e^{-aD}$ types of induction are needed.

Ultraviolet mutagenesis has not been put in the negative column because in the one system where we have full confidence in our work the number of revertants, R, per survivor, S, follows a relation $R/s = bD(1 - e^{-aD})$ and with this analysis, the value of a is in good agreement with that for the induction of protein X.

Our summary conclusion from this work is that all these phenomena are, in some way, influenced by the induction of the recA gene product. Most are directly and rather simply related to it. Those that are not either reflect a different endpoint in the analysis of the effect, or the need for an added effect of ultraviolet light beyond the induction of protein X. In this class is the separate "mutagenic lesion" in addition to the inducing lesion.

II. The action spectra for induction and the mutagenic lesion in WU3610-89.

This is described in Appendix 3, comments on a draft of a paper which stems from a presentation at the Burlington meeting of the American Society for Photobiology. A considerable body of data has been accumulated for the development of leucine revertants under the influence of monochromatic light. To a first approximation, a large part of the dose/response curve fits the relation we have quoted above,

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namely

Revertants per survivor = $bD(1 - e^{-aD})$.....(1)

At low doses this approximates to $R/S = baD^2$ and the value of the product ba can be found. At sufficiently high doses $1 - e^{-aD}$ becomes unity and the value of R/S becomes bD. One can then separate the two constants and plot them as a function of wavelengths. Figure 3.1 shows what results in this way. APPENDIX 3

The values found for the two constants are markedly different, b being quite small and a being much larger. Both appear to follow the absorption of nucleic acid, though not perfectly.

These experiments also show the existence of another mode of mutagenesis, not equally apparent at all wavelengths.

III. Weigle-reactivation for λ phage damaged by ionizing radiation.

The original observations on W-reactivation were made with UV damaged phage. It has also been possible, as reported earlier, to observe this phenomenon for phage damaged by ionizing radiation. This was reported on at the International Meeting for Radiation in Tokyo, this year. Phage can be inactivated under vacuum by fast electrons, by X-rays with broth protection, by X-rays with only buffer present. Of these, the most significantly reactivated is the vacuum inactivation by fast electrons which probably corresponds to direct action, in the anoxic state, on the DNA of the phage. It is of interest that W-reactivation of UV damaged phage shows to a lesser extent in uvr⁻ strains (Rothman, Clark et. al.) an observation which we confirm. However, the W-reactivation of phage damaged by fast electrons seems to be normally reactivatable in a uvr⁻ strain. We are actively following up this preliminary observation. APPENDIX 4.

IV. Induction phenomena in recBC and recF mutants.

The celebrated observation that a strain of E. coli which is recA⁻ and uvr⁻ is now rendered incapable of forming colonies at a UV dose which produces one

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dimer per genome and so is ultimately sensitive, has been matched by the observation that recB recF uvr strain is equally sensitive. This suggests that the more specialized mutational deficiencies in recB and recF together add up to the effect of a deficiency in recA. We have confirmed this finding of Rothman and Clark by observing the colony forming ability survival curves for UV damage for recA and recB recF strains. At low doses, reaching 10% survival the two are the same. At higher doses the recA strain is more sensitive. This has led Kazanis to look at the induction of protein X by UV and ionizing radiation in recB and recF and also recB recF strains. The study involved dose/response observations and also time-course observation. The results are very informative. The two strains behave differently. The development of protein X in the recB mutant requires considerable doses, more like the wild type strain AB1157. The appearance of the recA gene product is much more rapid in time after the insult from the UV. On the other hand, for recF cells the recA gene product is elicited at quite low doses and its appearance is delayed, with a very small development at times up to 25 minutes, in contrast to almost full development by that time in the wild type and recB strains. These experiments suggest that there are two modes of induction. In the recF strain the enzyme exonuclease V is fully active. It has the property of recognizing a signal to degrade, which is apparently a short single strand region, and then degrading for a long stretch, which can be the entire genome. This degradation process develops oligonucleotides, which can degrade further to nucleotides. Among these products is probably an effector molecule which acts to activate the recA gene product at its basal level, an activation which causes the inactivation of the lexA gene product, the repressor of recA. Small doses would cause considerable yield of the effector molecules but they would take time to accumulate to the needed concentration. Thus we see induction at small doses but taking time to express. The recBC mutant, on the other hand, has no exonuclease V activity and there is very much less DNA breakdown. Some other method of induction, not requiring a specific degradation

product is suggested as taking place. Our current hypothesis is that single strand regions of DNA which can accumulate in the absence of exonuclease V, can bind a few molecules of protein X and if the binding is to the right nucleotide sequence the bound protein X becomes activated. Collisions by lexA gene product protein molecules with these activated protein X molecules can result in the accumulation of inactive recA repressors. To achieve this requires a relatively large number of gaps and so high doses. On the other hand, collisions are rapid in a small cell and after that much damage there should be a relatively short waiting period before derepression of recA is appreciable. APPENDIX 5

V. Coordinate induction in recBC and recF mutant cells.

In parallel with the above work studies of other induction phenomena have been made. These are complicated by the fact that the absence of exonucleaseV removes the possibility of observation of inhibition of post radiation DNA degradation, and for not simple reasons, no induced radioresistance is seen in recBC mutants. Nevertheless the phenomena given in Table 2 have been observed. This is taken from a report to the American Society for Photobiology in June 1979.

Table 2

Dose/response relations for various phenomena compared with those for protein X in recBC and recF mutant strains.

Function	recF	recBC
Protein X	50% normal low doses delayed expression	75% normal high doses rapid expression
Induced inhibition of DNA degradation	+ readily saturated	not observed
Induced radioresistance	+ weak response	very slight or none
Induction of λ	normal	normal needing higher doses
Septum inhibition	normal at low doses	normal needing higher doses

It can be seen that for these two rather different strains, the induction of other phenomena relates to that of protein X about as expected. APPENDIX 6

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VI. The action spectrum for the induction of λ.

Four methods for observing the induction of λ in strain K12λ have been worked out. They can be applied during one experimental test without over taxing the worker. They are: the observation of infectious centers by plaque assay; the observation of free phage after the induced cells have undergone lysis; the observation of the production of endolysin after induction and the measurement of the difference in survival of colony forming ability between lysogens and non-lysogens. All of these are subject to some criticism, but each one has strong points and they can be used to supplement each other to gain a definitive result.

Preliminary action spectra have been obtained and were shown in last year's progress report. The completion is expected by the end of October.

VII. The observation of septum inhibition.

The technique developed by Brian Robinson for the observation of septum inhibition in its relation to dose is now routinely used. It is described in APPENDIX 7, the draft of a paper to be submitted to Photochemistry and Photobiology, and already reported at the Atlanta meeting of the Biophysical Society. This work has been expanded to include observations of septum inhibition in recB and recF cells plus the double mutant recBrecF. All three show induced septum inhibition: it is least in the double mutant which also induces less protein X.

VIII. The change in the pattern of UV mutagenesis when previous induction by nalidixic acid has been used.

Nalidixic acid is a potent inducer of protein X and this is believed to be associated directly with the "error-prone repair" system which causes pyrimidine dimers to be converted into altered base pairs, thus constituting a mutation. Nalidixic acid is not, itself, believed to be a very strong mutagen. This system has been under study by Anne Coleman with results described in

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APPENDIX 8. There is undoubtedly a region of UV dose in which the yield of revertants per survivor is more nearly linear with dose, as would be expected if the "error prone repair" system were induced. However, at both the low and the high dose range there are incomplete findings and these are being studied. This work is of some interest because, working with tryptophan revertants Doudney (Am. Soc. for Photobiology 6th meeting Abst WAM-D3 1978) has reported that the linearization after nalidixic acid induction does not show in the uvr$^-$ case. Our work suggests that it can.

IX. Effect of UV in the frozen state at -79°C.

This work was reported at the San Juan meeting of the American Society for Photobiology. A paper to be submitted to "Photochemistry and Photobiology" is included. APPENDIX 9. It is found that "frozen UV" is an excellent inducer of radioresistance and inhibition of post radiation DNA degradation. It is also found that freezing the K12λ lysogen is followed by very low recovery.

X. The observation of induced repair in Chlamydomonas.

Using pre-treatment with UV followed by cycloheximide to block protein synthesis Richard Page looked to see whether the sensitivity of colony forming ability to ionizing radiation has changed. In three experiments no real evidence of the development of radioresistance could be seen. We therefore tentatively conclude that as far as nuclear controlled operations are concerned Chlamydomonas does not show induced repair. It is possible that it is to be found in chloroplasts and since mutations are produced in that organelle and these should obey the induction-plus-mutagenic lesion method of being developed, such action is being looked for. There are no definite results so far, except that mutations have been observed. APPENDIX 10.

XI. Additional interesting observations.

We have several additional observations. One is the clear evidence that the λ lysogen of AB2470 (recB) is more resistant to both UV and X-rays than the

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non-lysogen. This differs from the ususal observation which is that the additional mode of death, by cell lysis, causes additional sensitivity. The reason can be found in comparison with other work in which temperature sensitive λ lysogens have been found to be less sensitive to UV after temperature induction, particularly if the temperature is returned to normal before λ is fully induced. Since our observations don't involve this pulse of derepression, we feel that somehow there must be transcription of some λ genes, notably red and gam which control an exonuclease and an inhibitor of it, respectively, taking place. This provides a secondary system for recombinational repair, diminished in the recB strain. Whether this transcription is a normally present phenomenon, or follows the absorption of the radiation, is not clear. In any event, the fact that some λ genes are expressed without the whole of λ, does make it likely that the induction of λ involves multiple processes and also that some binding of repressors may not be formidably tight.

Another observation has been that the inducible inhibitor of post radiation DNA degradation can be saturated by damage to the DNA. This suggests that it acts by binding to the DNA. It also suggests that the whole of the large amount of protein X (2% of all proteins) can not be available for this binding. Quantitative studies of the saturation should make it possible to estimate the number of inhibitor molecules present. A first estimate suggests about 5% of the total protein X.

We have found that the time delay in the induction of protein X in recF strain cells is somewhat matched by a delay in the induction of uvr^- cells and these further have the interesting property that the production of protein X continues for quite long times after the original induction. We are currently following this up by making a comparison between the retention of two induced repair phenomena, septum inhibition and induced radioresistance, with the amount of protein X produced. So far we have only observed the processes in the wild type, but it is clear that there is a separation between the various

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induced functions. This forms something we wish to study further.

A fourth observation, which we hesitate to report, for it contradicts what seems to be careful work, is that in strain AB1886, which is uvr$^-$, we observe little or no Weigle-reactivation. Since this is readily observed in strain AB1157, the wild type, we find the result disquieting. It is possible that our assay system is not the same as that of other workers. This is an area in which we want to do further work.

April 5/6 1979, revised July 13 1979.

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APPENDIX 1

SUMMARY OF THE PRESENT STATE OF KNOWLEDGE OF INDUCED REPAIR.

The acceptance of the phenomenon of induced (S.O.S.) repair can almost be said to date from the Gainesville Conference April 4-6 1975. This is roughly four years ago. The finding that radiation itself greatly enhanced an agent which also modified the effect of radiation, seemed to offer great potential for advancing the understanding of radiation action on cells. In this appendix an attempt will be made to assess the present position of knowledge regarding induced repair and to see to what extent the potential is as great as was thought.

Almost all the work of compelling significance has been done in Escherichia coli. The reason for this has been the discovery, and characterization, of genes that control aspects of response to radiation of various kinds, notably ultraviolet light. Gradually the character and mode of action of these genes have been elucidated, sometimes in detail. The best studied is the process of excision repair, controlled by the uvr genes. Cells which lack the ability to perform excision repair, however, are not maximally sensitive. Another set of genes, the rec genes control a form of repair which involves synapsing between DNA duplexes, hence the term "recombination". One gene, recA, is very potent in this system, and a cell which is both recA$^-$ and uvr$^-$ does have maximal sensitivity: that is to say, one pyrimidine dimer in the genome is able to stop colony forming ability.

Associated with the active presence of the recA gene are found a number of apparently unrelated phenomena. The list of these grows and is already over 10 in number. A common characteristic of these phenomena is that they develop after the administering of UV light, or ionizing radiation or the exposure to nalidixic acid, or mitomycin C. The phenomena only develop if protein synthesis is allowed to occur. These agents are called inducing agents, and the phenomena are considered to be due to induced repair, also called "S.O.S." repair. One task

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we have assigned ourselves in our laboratory is to examine a number of these phenomena as induced by the absorption of monochromatic UV light, to see whether they are all elicited in precisely the same way, as they should be if they are all simply due to the derepression, in some manner, of the recA gene and the consequent transcription of the gene product. This work continues, but one conclusion we can state is that four of the seven phenomena we have studied go so closely together that we don't doubt that they are due to the same process. Three others are not so simple. One of these, induced radioresistance, may differ only because the endpoint measured, which is the survival of a colony, could result from the induction of only one of several genomes present in one envelope. The other two: mutagenesis and the induction of prophage, seem clearly to be closely involved with the derepression of the recA gene, but have an added requirement which must be provided by the radiation. Mutagenesis requires the pre-mutational lesion and λ induction requires a second induction as well as that of recA. Thus the coordinate nature of the effects due to the derepression of the recA gene, as deduced from our work, is very likely. We believe this kind of careful work is needed to give the hypothesis of induced repair the support necessary to make it useful to others in the interpretation of radiation action.

Important Aspects of Induced Repair

There are three major aspects of induced repair which need study and clarification. The first is the nature and scope of its action. The second is what signals induce it? The third is how general a phenomenon is it?

Knowledge exists on these three aspects to quite different degrees. For none of them is it nearly satisfactory.

Nature and scope of action of induced repair

In cells which have the needed genetic complement, namely they are $recA^+$ and lex^+, the following phenomena are now recognized. In response to several inducing agents, notably UV light, nalidixic acid, ionizing radiation, mitomycin C and, where applicable, thymine starvation, or temperature shift, cells develop,

in about 40 minutes, a large amount of a protein which is not readily seen in uninduced cells, though it is undoubtedly there. This was formerly called "Protein X", so named by its discoverer, Inouye. It has now been identified as the recA gene product and is being referred to in that way. We shall use "precA" as an alternative to "protein X". The amount of precA can reach 2% of the total cellular protein. Its molecular weight is around 39,000.

Induced cells show marked differences from uninduced. The list of these differences includes septum inhibition, inhibition of post radiation and other DNA degradation, Weigle-reactivation, linear-response UV mutagenesis, increased radioresistance, diminution in respiration and an increased ability to perform long patch repair. The work we have quoted above strongly suggests to us that these differences are closely related to the induction of protein X, though there can be additional requirements superposed on the induction.

The properties of precA are only beginning to be described. One important facet of its action is the ability to become "activated". In combination with single strand DNA it is able to accelerate the reassociation of homologous stretches of DNA. To do this, the single stranded region must have the homologous strand present. Also, when activated by single strand DNA, precA can catalyze the hydrolysis of ATP. These two properties, discovered by Weinstock, McEntee and Lehman (1979, PNAS 76 126-130), suggest the usefulness of precA in recombinational repair. In addition, by a mechanism still not described, the combination of protein X with single stranded regions of DNA inhibits DNA degradation. This degradation is necessary in recombinational repair, though exactly why is not clear. It is also necessary that the DNA degradation be held in check and not remove needed DNA. This seems to be the function of the inducible inhibition of DNA degradation, and protein X plays a part in it. We find the induction of protein X to be coordinate with septum inhibition, suggested by Satta and Pardee (1978 J. Bact 133 1492-1500) to be an action of protein X. How protein X achieves this is not yet known. It may do so in the unactivated form. Probably the halt in respiration and

the inhibition of the formation of the septum are linked, but there is no evidence that we know to back up this hypothesis. Most intriguing is the potentiation of the pre-mutagenic UV lesion. Radman has suggested that this is due to the inhibition of the 3' - 5' proofreading function of polymerase A. In our view more evidence is needed to support this idea and the whole mutagenic action needs much more work. The conferring of radioresistance (and probably also other kinds of resistance, including thermal) is not understood. It could most simply be linked to septum inhibition, but this is contradicted by the existence of nearly normal septum inhibition in recB$^-$ cells, with no radioresistance apparent. Our suggestion is that it is a part of the function of inhibition of DNA degradation. Exactly, how, we do not know.

To summarize, there seem to be three processes resulting from the derepression of recA which need major consideration. First, the ability to prevent the formation of the septum at the normally scheduled time. Second, the ability to combine with single stranded DNA to prevent DNA degradation. Third, the potentiation of synapsing between homologous regions of different duplexes. It is probable that all the so-called S.O.S. functions can be deduced from these three.

The signal to induce

This has become much harder to define as our knowledge has increased. In the early days of a correlation with the cessation of DNA synthesis as produced by the effective inducing agents, the signal was thought to be precursors of DNA which piled up and gave the signal as effector molecules. This, which is not often suggested today, may still be the signal. One cogent reason against this was advanced in consideration of the low level of spontaneous induction of lambda. This is about 1 in 10^5 and implies a tight binding between the repressor and the DNA, so tight that the equilibrium constant is far over to the bound side. To reverse this with a concentration increase of something already present to a moderate concentration, would need a very great increase in precursors. Earlier observations in our laboratory showed a measured increase of about a factor of

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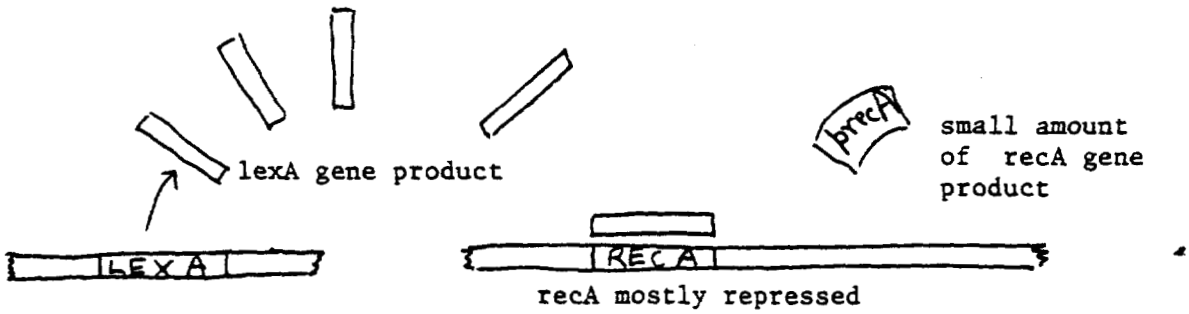
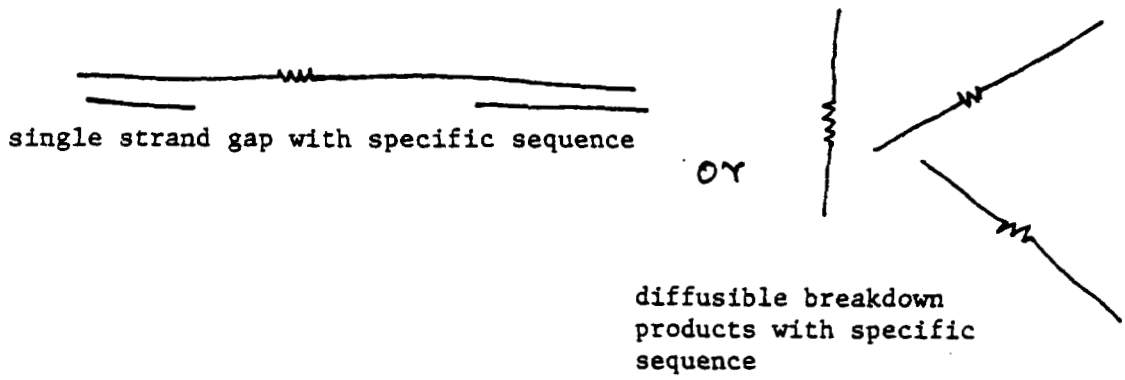
REPRESSED CONDITION



DNA DAMAGE



ACTIVATION AND DEREPRESSION

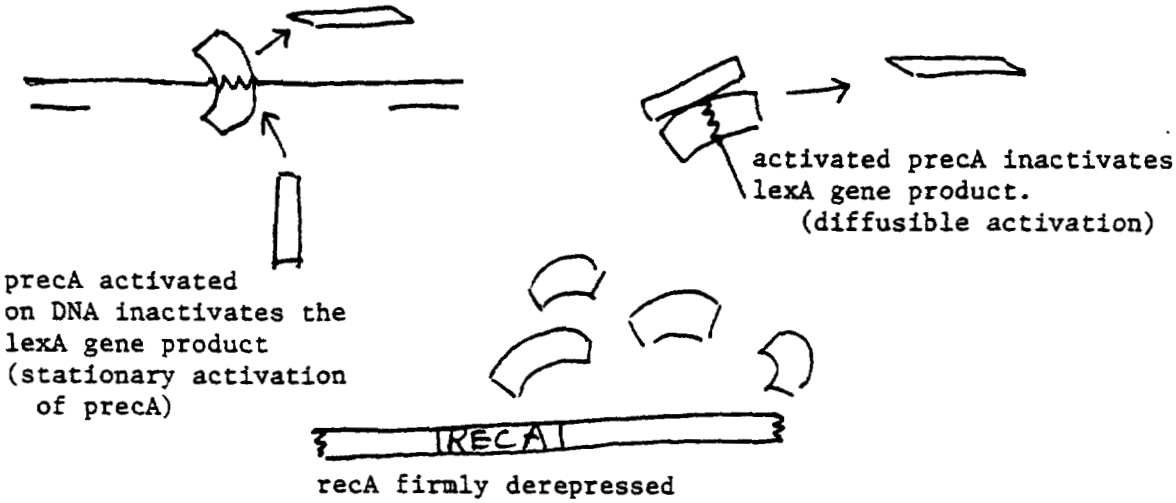


Figure A1. Illustrating the suggested way in which the lexA, recA gene products interact to give firm derepression of recA when there is DNA damage. Two methods by which a specific sequence on single strand DNA can act to make the recA gene product able to alter the lexA gene product so that it no longer acts as the repressor of recA.

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10, not enough to shift the equilibrium. However, if it be supposed that the induction of lambda is a multistep process, for which there is increasing evidence, the spontaneous induction of one of these steps could be much greater, meaning the binding of the repressor is not so tight. A factor of 10 could then have some influence. Precursors could then be thought of as candidates for the inducing signal.

The most commonly made suggestion today is that oligonucleotides are the effector molecules which ultimately cause the derepression of the recA gene. This is plausible, for in most cases, the agents which cause induction also cause some DNA degradation. Two lines of evidence speak against this idea. One is the experiment of Little and Hanawalt who introduced unrestricted λ into a $recA^+$ cells without inducing derepression. The restrictive degradation of the phage should have provided the signal for induction. The second line of evidence is that in $recB^-$ cells, where there is very much less DNA degradation, there is normal induction of septum inhibition and of λ.

One very significant fact about the induction process is the great amount of precA which is produced. In order for a derepression to be as firm as this there has to be some means of keeping the operon open. We suggest the scheme shown in Figure A1 to achieve this. This scheme is based on reading the current literature, from conversations and our own observations and discussions. The repressor for the recA operon is produced by the gene lexA. This produces a diffusible protein which binds to the recA operator and cause repression. The binding, in the non mutant lexA case is not so tight that a small amount of protein X is not formed. This is without any action of the lexA gene product unless it becomes activated by some consequence of DNA damage. The DNA damage is suggested by us to be of two kinds. The first is the presence of relatively long single stranded gaps containing a special base sequence. This can bind and hold a precA molecule which becomes activated while held. This we call stationary activation. The other kind is a diffusible breakdown product which also has the specific base sequence and which can bind to precA and activate it.

There are now two kinds of action possible. One is the collision between the lexA gene product and the stationary activated precA molecule, a collision which results in the inactivation of the recA repressor. The other is collision with the diffusible activated precA molecule, which also results in the inactivation of the recA repressor. In either event, the result is inactivation of the lexA gene product and a firm derepression of the recA operon, which remains firm so long as the concentration of activated precA molecules is high enough to take care of the newly synthesized repressors. This mechanism, while hypothetical, can fit all data we know of, though it leaves a great deal unanswered as regards the other actions of the activated (or not) precA molecules.

The recBC and recF "pathways"

The dual character of the activation of protein X has been suggested to us by work by Kazanis on the induction of protein X in recBC and recF cells. Rothman and Clark have shown that the double deficiency in recBC and recF is equivalent to deficiency in recA. The cell can manage without the DNA degradation properties of exonuclease V, the recBC gene product, if it can call on the recF gene product, thought to be an endonuclease. If this last is not there, then the cell becomes unable to execute recombinational repair and has high sensitivity. Accordingly, if one looks at the production of protein X after UV induction, it is possible to separate, to some extent, the effect of the presence of the gene products of the two mutants. Thus in recBC there will be no exonuclease V and therefore a greatly reduced degradation of the DNA, and consequently a greatly reduced number of potential oligonucleotide effector molecules. There will be more gaps not degraded out. On the other hand, in recF cells there will be full activity of enonuclease V and plentiful oligonucleotides.

It is found that both mutants produce less precA than the wild type and they do so with different dose-response relations for UV induction. There is also a difference in the time of development of the protein X. recF cells are induced at quite low doses and the induction takes about an hour to become maximal.

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recBC cells are induced maximally by higher doses, but the time sequence of induction is such that they become induced sooner, with the maximum yield in about 40 minutes. Thus two different "pathways" to induction can be seen. The present working hypothesis in our lab is that the recF cells induce by the diffusible activation method, one of the DNA degradation products being the effector. The time for these to be produced by exonuclease V accounts for the slow induction. The fact that exonuclease V operates to degrade a long stretch of DNA once it starts accounts for the high sensitivity. On the other hand, the $recB^-$ cells must induce by some other method, requiring more dose, but being more immediate. We suggest that what we call stationary activation is the major means of activation in this case. In terms of dose it is less efficient than an enzymatically produced diffusing effector molecule and would need more dose: however, it would act faster when such a dose were given.

Clearly more work is needed to establish the nature of the signal to induce and it is likely that it will prove to be several signals.

The generality (or otherwise) of Induced Repair

At the Gainesville conference, it was widely felt that induced repair will be found in a majority of systems. It was suggested that many split dose effects could be explained in this way. It was freely suggested that the induction of λ, the first phenomenon to be linked with induced repair, is a model for cells to become malignant. In the four years which have elapsed since then there is some question as to the validity of this easy generalization.

It is most important to consider. If, indeed, carcinogenesis and mutagenesis are very closely linked, as is being freely said today, then the process of mutagenesis, which needs the "error-prone-repair" process, believed to be an inducible "S.O.S." component, will lead us to the conclusion that cell transformation is also something requiring the S.O.S. component.

There seems to be very little doubt that in B.subtilis there is a close counterpart to the induced repair system as observed in E.coli. Indeed it is

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probably a factor in rendering the cells transformable by transforming DNA. In a marine pseudomonad, neither induced radioresistance, nor induced inhibition of DNA degradation could be observed. In Micrococcus luteus there is no evidence of induced radioresistance. This could not be found in Chlamydomonas. There are reports of induced "error-prone" repair in yeast. There is some suggestion that the inducible inhibitor of DNA degradation may be found in the yeast mitochondrion.

As far as mammalian cells are concerned there is solid evidence that the process of Weigle-reactivation, one of the first induced repair phenomena to be observed, is seen for several viruses and several cell types. The necessary studies of protein synthesis inhibition have been done and it can fairly be concluded that some induced repair processes must be present in mammalian cells. Other phenomena are not so easily put into the demonstrated category. A short review by McCormick and Maher makes this clear. Thus the situation which is very important to clarify: the extent to which the findings in E.coli can be transferred to human cells is not at all clear. At the moment it is par excellence an area for prejudice one way or the other.

This situation is momentarily inevitable. The quite interesting findings in E.coli are phenomenologically definite, but not interpreted in any satisfying detail. When they are, it will very probably be seen where they can apply in other systems. For example, induced repair may be found in the organelles that have resulted from symbiosis with microorganisms. One temptation we have encountered is to say that since septum inhibition is one of the prominent manifestations of induced repair, and since a rod shape permits the packaging of multiple genomes which are needed for recombination, the most likely organisms to show induced repair will be rod shaped. Mitochondria are often rod shaped: one feels like predicting that the manifestation of induced repair in mammalian cells will be found in mitochondria. This idea, if it should prove right, might be trivial to the broad health picture, but in the long run of biological understanding

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it may be interesting and important. Obviously research has to continue.

Low dose effects

One interesting and potentially important consequence of the nature of induced repair concerns effects at very low doses. One characteristic which shows several times in studies of induced repair is the all-or-nothing character of induction. Induction follows a Yield = $(1 - e^{-X})$ type of relation, (X is proportional to dose) a relation which is very easily explained by the supposition that a given dose either induces a cell or does not. At high doses it can be supposed that all cells are induced, as expected from the formula above. These cells will have all the properties of induced cells, notably radioresistance, ready conversion of pre-mutational damage into mutations, and readiness to induce prophage. However, at low doses the cells will be found in two populations: one which has been irradiated and has not been induced, and one which has. The former form a group which is sensitive but which can not readily mutate nor induce prophage. The latter will be resistant, but mutable and phage inducible. If, then, we take data at high doses, for sensitivity, mutability and phage inducibility and extrapolate from these data to low doses, our conclusions will only be correct for a part of the population. A part will exist which is quite different and about which quite misleading conclusions may be drawn. If it should be true that this is, indeed, occurring, and if there is induced repair of the same type in mammalian cells, then there is grave danger in attempting to extrapolate from moderate to high doses to low doses. We believe that this secondary consequence of the nature of induced repair must be examined with some urgency for it can easily be seen to have a bearing on the health hazard estimates for very low doses to human populations. We are seeking ways to pursue preliminary work in this area which we have already done, to make the situation, at least in E.coli, definite.

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Concluding remarks

The relatively small number of geneticists, biochemists and radiation biologists who have studied induced repair in the past five years or so have made considerable advances. It will be clear from the above pages that the main achievement has been to bring the process of induced repair out of the interesting and suggestive stage into that of having definite characteristics and a specific gene product at work in some way in generating those characteristics. The clear and definite understanding of how it is elicited and how it functions is not yet available.

April 4 1979, revised July 14 1979.

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APPENDIX 2

COORDINATE BEHAVIOR OF ULTRAVIOLET LIGHT INDUCED REPAIR (S.O.S.) PHENOMENA IN 3 STRAINS OF E.coli

D.J. Fluke, Deno Kazanis and E.C. Pollard

INTRODUCTION

Separate discoveries have been made of at least 10 phenomena in E. coli which are induced by various agents, notably UV radiation, nalidixic acid, ionizing radiation, mitomycin C and, where applicable, thymine starvation or temperature shift. Induction needs the $lexA^+$ and $recA^+$ genotypes. The 10 phenomena are: Induction of λ (1, 2); W-reactivation and mutagenesis (3); filament formation and septum inhibition (4, 5); induced inhibition of post radiation DNA degradation (6); induced mutagenesis (7); induced radioresistance (8); protein X (9); "long patch" repair (10); repair of double straind breaks and crosslinks (11).

One important phenomenon is the production of "protein X', so named by its discoverer, Inouye, which, in about 40 minutes of incubation after induction develops to about 2% of the total protein in the cell (12). This has been identified as the recA gene product (13).

If all of these phenomena are manifestations of the derepression of recA and the formation of its gene product, then the dose-response behavior of all 10 to a single inducing agent should show considerable similarity, if not identity. We have called this behavior "coordinate behavior" (14). To test coordinate behavior we have used ultraviolet radiation as the inducing agent. It is suitable because it is known to be absorbed in nucleic acids, produces a known effect (pyrimidine dimers) and by using absolute dosimetry we can be quite sure of the actual amount of exposure in every case. The phenomena we have studied and report on here have been: protein X; induced inhibition of post radiation DNA degradation (IIprd); induced radioresistance (IRR); septum inhibition (SI); W-reactivation; UV mutagenesis; the induction of λ phage. We have worked with 3 strains, all $lexA^+recA^+$: AB1157

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(uvr$^+$) AB1886 (uvrA$^-$) and WU3610-89 (uvr$^-$).

We find most, but not all, of the phenomena are coordinate. Explanations for the deviations are suggested.

MATERIALS AND METHODS

UV exposure

Ultraviolet radiation is given with a water prism double monochromator with a high pressure mercury arc serving as the entrance slit. Four 12" reflecting mirrors are used for collimators and telescopes. The intensity of 265nm light at the output where the exposure is given is roughly one watt. Exposure was given in growth medium (chilled) in a silica dish with incident and transmitted light intensity measured. The average energy is calculated (15).

Cell strains

AB1157 and AB1886 were obtained from the E. coli genetic stock center. WU3610-89 as obtained from Evelyn Witkin via Dr. S. Person.

Growth medium

The cells were grown in C-minimal medium (2gNH_4Cl, 6gNa_2HPO_4, 3gKH_2PO_4, 3gNaCl, 120mg$MgCl_2$, 60mgNa_2SO_4 with 5g glucose and 2g casamino acids per liter). The glucose was autoclaved separately and then mixed. This is called "casaC" medium.

Revertant agar

C minimal salts, less the $MgCl_2$, with 12g/liter agar were autoclaved and separately sterile tyrosine (for WU3610-89) or proline, threonine, arginine and leucine at 20mg/liter each (for AB1886), sterile $MgCl_2$ at 120mg/liter glucose at 5g/liter and 30ml casaC medium added.

Induction of λ

Two methods were used for the observation of the induction of λ. The first was the observation of the number of infectious centers at 40 minutes after induction, the second was the observation of free phage 120 minutes after induction. In this latter case a burst size of 100 was assumed in figuring the number of induced cells. This is based on a fair number of trials and represents an average. Plating

was performed on bottom layer agar consisting of 23g nutrient agar and 5g of salt per liter, with a top agar layer of 7.5g nutrient agar + 5g salt per liter. We used strain Q1 as indicator bacteria, though on occasion we used AB2470 (recB$^-$) because this gives large easily visible plaques. The indicator bacteria was grown on slants and then suspended in 5ml broth immediately before plating. 2 drops were used to a tube. The top layer agar has to be carefully managed. If it is too cool there are no plaques and if it is too hot the infectious centers are damaged. We found it necessary to work between 42°C and 44°C and also to keep the top layer agar tubes in the 42°C bath no longer than 5 minutes.

Protein X

We used a technique similar to that reported elsewhere (9). We found we had more readily observed bands with an 8 to 20% gradient gel and we found the protein X band regularly appeared with staining. For quantitative work we used a radioactive label with ^{35}S. We cut out the band of protein X, dissolved it in 30% H_2O_2 and counted it in a scintillation counter. We also cut out the rest of the gel, dissolved it and counted it. In this way we were able to measure the ratio of the amount of protein X to the amount of newly synthesized protein in the remainder of the gel.

DNA degradation

Cells were labeled with 200mg/ml deoxyadenosine and 1 mCi per ml of ^3H-thymidine with 1mg/ml cold thymidine. We observed the degradation by putting the cells into 5% cold trichloroacetic acid (TCA), keeping the mixture cold for 30 minutes, centrifuging and counting the supernatant. The total activity was measured by putting some samples in 10% TCA at 95°C for about an hour and then counting the supernatant.

W-reactivation

This is described more fully in a later appendix. λ phage, inactivated to about 10^{-3} to 10^{-5} were attached to cells which either had or had not been given various doses of 265nm light. The phage survival was then observed and the W-

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reactivation factor measured.

Induced radioresistance

Cells were grown to about 2 X 10^8 per ml. They were then irradiated with various doses of 265nm light. After this treatment they were incubated at 37°C for 40 minutes, given 50 micrograms per ml. rifampin, incubated 10 more minutes and chilled in ice. They were then subjected to five increasing doses of 50kvp X-rays from a Picker X-ray machine with 50mg/cm^2 aluminum filtration. They were then routinely diluted and plated for colony forming ability.

Septum inhibition

This is fully described in a later appendix. After 90 minutes incubation the cells were observed in a microscope under "High dry" conditions and photographed. The resulting pictures were then analyzed by length and the fraction exceeding the normal length considered as septum inhibited.

UV mutagenesis

The basic procedure has been previously described (15). In these experiments we routinely plated the unirradiated samples at two or three dilutions. This enables an estimate to be made of the number of existing mutants as opposed to spontaneously developing mutants. If the number is excessive then fresh cultures can be grown, a procedure we did not find necessary. The colony survival was determined on revertant agar.

RESULTS

Since the whole basis of this study is comparative we have followed the procedure of plotting each measurement of dose-response yield and then estimating the fraction of maximum for each dose. Several trials were made in each case and we are presenting the average. For strain AB1157 the results shown in Table 1 were obtained. In the table are presented the averaged fractions of maximum for each dose. On the left is the fractional yield of protein X (which we also call precA) at 60 minutes of incubation. We then give the fractional yield, at the same doses, for five other phenomena. The ratio of the yield of these five phenomena to the yield

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of precA is also given. If the phenomena are coordinate with the derepression of recA then this ratio should be unity. A striking deviation indicates that some additional factor is present. Something can be inferred from the character of the deviation and this we discuss.

In order to see how the various phenomena behave it is useful to look at a plot of the data in Table 1. It can be seen that none of them are an exact fit, something not really expected. On the other hand the agreement between W-reactivation, septum inhibition and induced inhibition of post radiation DNA degradation is such that all of them approach the ratio unity at relatively small doses and do not deviate much from it. In contrast the induction of λ shows a start at zero and a steady rise, itself not a linear rise. It can hardly be claimed that the induction of λ is coordinate with precA. An intermediate case is induced radioresistance which shows a ratio rising above unity and falling again. The feeling suggested is that somehow the endpoint of colony forming ability distorts the appearance of the data while not necessarily being an uncoordinate phenomenon.

When the figures of Table 2 are plotted the appearance is as in Figure 2. It is at once apparent that for all the phenomena the dose range is curtailed, now covering about $3J/m^2$ rather than 15. It is also clear that there is more dispersion in the way the various phenomena behave in relation to the development of the recA gene product. Two of the phenomena seem to be genuinely coordinate: induced inhibition of post radiation DNA degradation and septum inhibition. A third would be expected to fit in, but since we found W-reactivation to be present to so small a degree we were unable to include it. The ratio found for histidine revertants behaves most interestingly. At very low doses the number of revertants per survivor remains constant, something reported at the Photobiology Society meeting by Sargentini and Smith, then it rises proportionally to the yield of precA and at higher doses begins a sharp rise. The region from 0.5 to $2J/m^2$ can be thought of as coordinate, for mutagenesis presumably requires the presence of a premutagenic lesion as well as the induction of the induced

TABLE 1

The averaged fractional yields for precA (protein X) and five other manifestations of induced repair.

Strain AB1157 (wt)

DOSE	precA	W-reactivation	ratio	IRR	ratio	SI	ratio	IIPRD	ratio	λ-ind	ratio
0.5	0.175	0.128	0.74	0.13	0.74	0.16	0.91	0.18	1.03	0.0018	0.01
1.0	0.310	0.236	0.76	0.28	0.90	0.26	0.84	0.29	0.95	0.011	0.04
1.5	0.415	0.314	0.76	0.41	0.99	0.34	0.82	0.38	0.92	0.027	0.07
2.0	0.485	0.413	0.85	0.56	1.15	0.42	0.87	0.45	0.93	0.050	0.08
3.0	0.605	0.547	0.90	0.83	1.37	0.55	0.91	0.57	0.94	0.116	0.19
4.0	0.700	0.650	0.93	0.95	1.36	0.66	0.94	0.66	0.94	0.23	0.33
5.0	0.785	0.75	0.94	0.98	1.25	0.74	0.94	0.74	0.94	0.35	0.19
6.0	0.855	0.82	0.96	1.00	1.17	0.80	0.94	0.82	0.96	0.46	0.54
8.0	0.950	0.88	0.93	0.96	1.01	0.89	0.94	0.93	0.98	0.70	0.74
10.0	0.990	0.93	0.94	0.87	0.88	0.95	0.96	1.00	1.01	0.92	0.93

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TABLE 2

Data for the induction of six phenomena in strain AB1886 with the ratio to the yield of protein X (precA)

DOSE	precA	S.I	SI/X	IRR	IRR/X	IIPRD	II/X	λ	λ/X	R/S	R/S/X
0.2	0.16	0.32	2.00	0.75	4.69	0.13	0.81	0.05	0.31	25	151
0.5	0.47	0.52	1.11	1.02	2.17	0.44	0.94	0.23	0.33	80	170
1.0	0.63	0.83	1.32	1.00	1.59	0.72	1.14	0.61	0.97	235	372
1.5	0.70	0.95	1.36	0.98	1.40	0.93	1.33	0.92	1.31	470	670
2.0	0.84	1.00	1.19	0.94	1.12	0.96	1.14	0.98	1.17	810	970
3.0	0.93	0.98	1.05	0.86	0.92	0.98	1.05	0.77	0.80	2150	2310
4.0	0.96	0.96	1.00			0.99	1.03				
6.0	1.00	0.86	0.86			1.00	1.00				
10.0	0.95										

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repair system. Something else must be occurring above $2J/m^2$.

The induction of λ, as was the case for AB1157 shows a ratio close to zero for low doses and a sharp steady rise to a ratio near unity. Beyond this there is a fall. It is evident that ultraviolet light has actions in addition to the derepression of the recA gene product, as far as the induction of λ is concerned.

The behavior of induced radioresistance is more anomalous than in the case of strain AB1157. The ratio to the yield of the recA gene product rises very sharply at low doses and thereafter drops sharply. So far no simple interpretation of this has suggested itself to us.

In Table 3 the findings for strain WU3610-89 are shown. Again the dose-range covered is even less than that for AB1886. The ratios for septum inhibition and IIPRD are coordinate except at very low doses. Most striking is the remarkable proportionality to dose exhibited by the leucine revertants. This has been previously discussed (15) without the added feature of the comparison with the measured yield of the recA gene product. The leucine revertant yield has been studied more carefully than any other yield in our laboratory. We can say with some confidence that the number of revertants per survivor, R/S, obeys the relation

$$R/S = k_1 D f(D) \qquad (k_1 \text{ a constant; } D \text{ the dose})$$

where f(D) is just the same as the dose response relation for protein X.

The anomalous behavior of induced radioresistance shows again in a rather less marked manner than for strain AB1886. Again we have no suggestion beyond asking whether the endpoint observed may not be sufficiently different to introduce a bias.

DISCUSSION

The primary impression we receive from this work is that the seven phenomena we have looked at are, in some way, all closely involved with the derepression of recA.

In three cases: W-reactivation, septum inhibition and induced inhibition of post radiation DNA degradation, we believe that the behavior is coordinate and that experimental considerations are all that are involved in any differences. For induced

TABLE 3

Ratios as previously for strain WU3610-89

DOSE	Protein X	SI	SI/X	IIPRD	IIPRD/X	IRR	IRR/X	$\frac{R-Ro}{S}$	$\frac{R-Ro}{SX}$
0.1	0.19	0.28	1.45	0.19	1.00	0.39	2.05	15	80
0.2	0.34	0.49	1.42	0.46	1.35	0.72	2.11	63	190
0.3	0.53	0.61	1.15	0.64	1.20	0.98	1.85	130	243
0.4	0.62	0.68	1.10	0.70	1.17	1.00	1.63	215	350
0.5	0.71	0.75	1.05	0.74	1.04	0.98	1.29	320	450
1.0	0.84	0.87	1.03	0.86	1.02	0.89	1.05	960	1140
1.5	0.91	0.94	1.04	0.92	1.01	0.73	0.80	1290	1420
2.0	0.95	0.96	1.00	0.95	1.00	0.65	0.68	1840	1940
3.0	0.98	0.98	1.00	0.98	1.00			3000	3060

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radioresistance we suggest the specific endpoint of the survival of a colony may relate differently to the concentration of the recA gene product than the other phenomena do. For mutagenesis there are clearly cases where the induction of precA is needed, with a premutational lesion, proportional in number to the dose an added requirement. There are, however, also other mechanisms of production of mutations.

The induction of λ while also clearly requiring the derepression of recA must have an additional requirement for UV dose. What this second requirement is we do not yet know.

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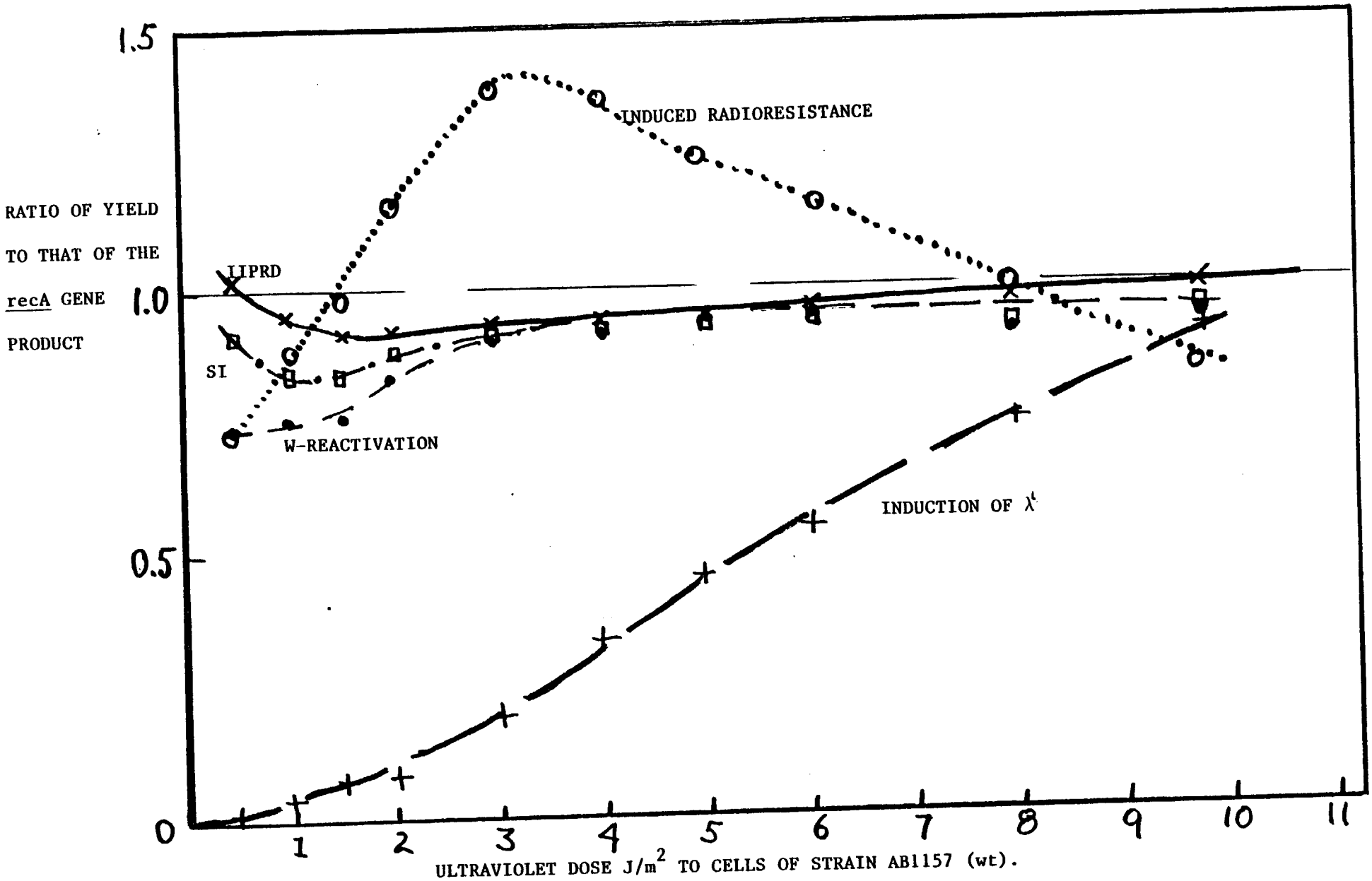

Figure 2.1 Comparison between the yield of various phenomena associated with induced repair and the yield of the recA gene product. Coordinate induction should yield a line uniformly at unity. The induction of λ clearly does not obey this requirement. Induced radioresistance is also anomalous, though not in the regularly rising way exhibited by λ induction.

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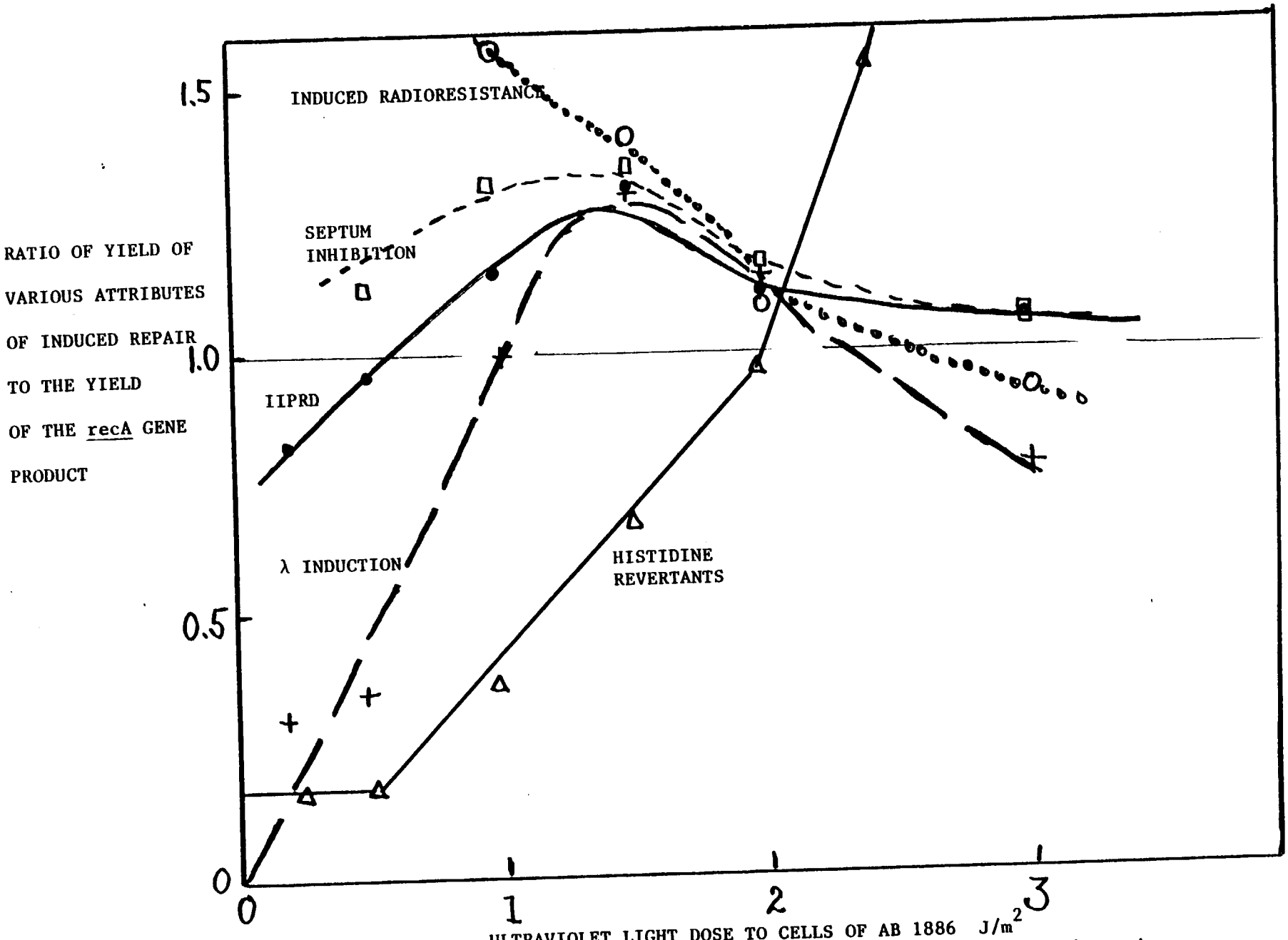

Figure 2.2 A comparison of the yields of various manifestations of induced repair in a uvr⁻ strain. The dose range is less and the agreement with the expected ratio of unity for coordinate behavior is markedly less good than for the uvr⁺ case. The major exceptions are nevertheless the same phenomena as observed in other cases.

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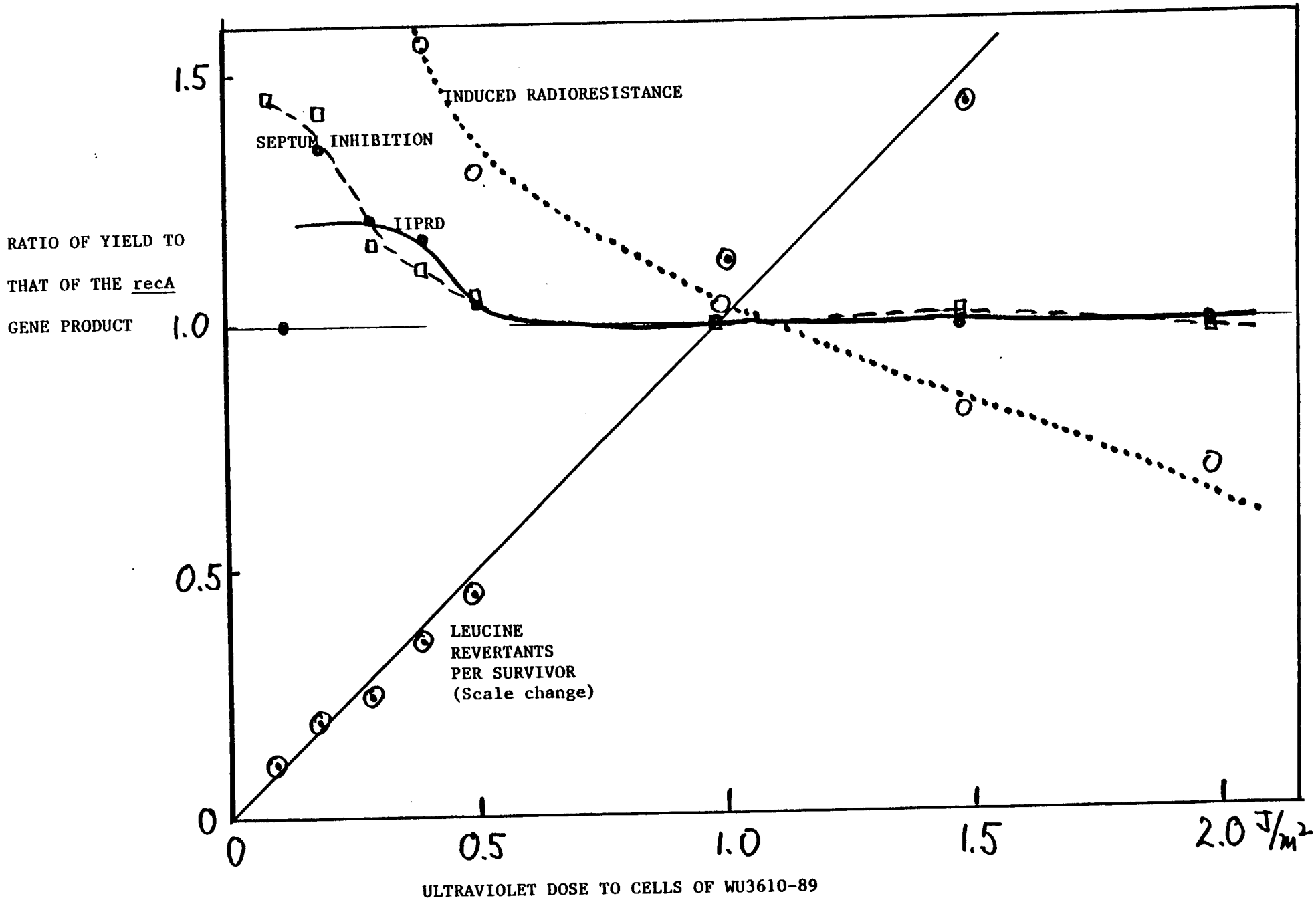

Figure 2.3 Comparison of the yields of various manifestations of induced repair with the yield of the recA gene product. The scale is different for the leucine revertants as there are approximately 1000 such at 1 J/m^2.

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APPENDIX 3

COMMENTS ON THE NATURE OF UV INDUCED MUTAGENESIS

D.J.Fluke and E.C.Pollard

A considerable part of our effort has been directed to an intensive study of leucine revertants as developed by ultraviolet light and, on occasion X-rays. For strain WU3610-89, we have satisfied ourselves that there are two components to the majority of UV induced mutagenesis: the induction of the induced repair system, which is essentially identical in character to the dose response relation for the derepression of the recA gene (see Appendix 2), and a premutagenic lesion. In last years progress report we gave an outline of a paper presented at the Burlington meeting of the American Society for Photobiology. There a method for analyzing the data according to the relation

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

where D is the dose and R/S is the ratio of revertants to survivors. k_1 is the sensitivity cross section for the premutagenic lesion and k_2 is the sensitivity cross section for the induction of induced repair, as far as we can tell identical with the sensitivity cross section for the induction of protein X the recA gene product. The method of analysis separated the two constants k_1 and k_2 and in Figure 3.1 we show approximate averages of the data plotted with the DNA absorbance spectrum through the data points. The fit is certainly suggestive, but it is by no means perfect and there is more than a suggestion that wavelengths more associated with protein absorption may be quite important.

There is clearly some significance in the disparity in the two cross sections. That for induction is somewhere around unity, whichagrees broadly with the finding that induction of induced repair phenomena in this strain is considerable at 1 J/m^2. Since this produces 55 dimers per genome the probability of one dimer causing induction is considerable. The value of k_2, the sensitivity

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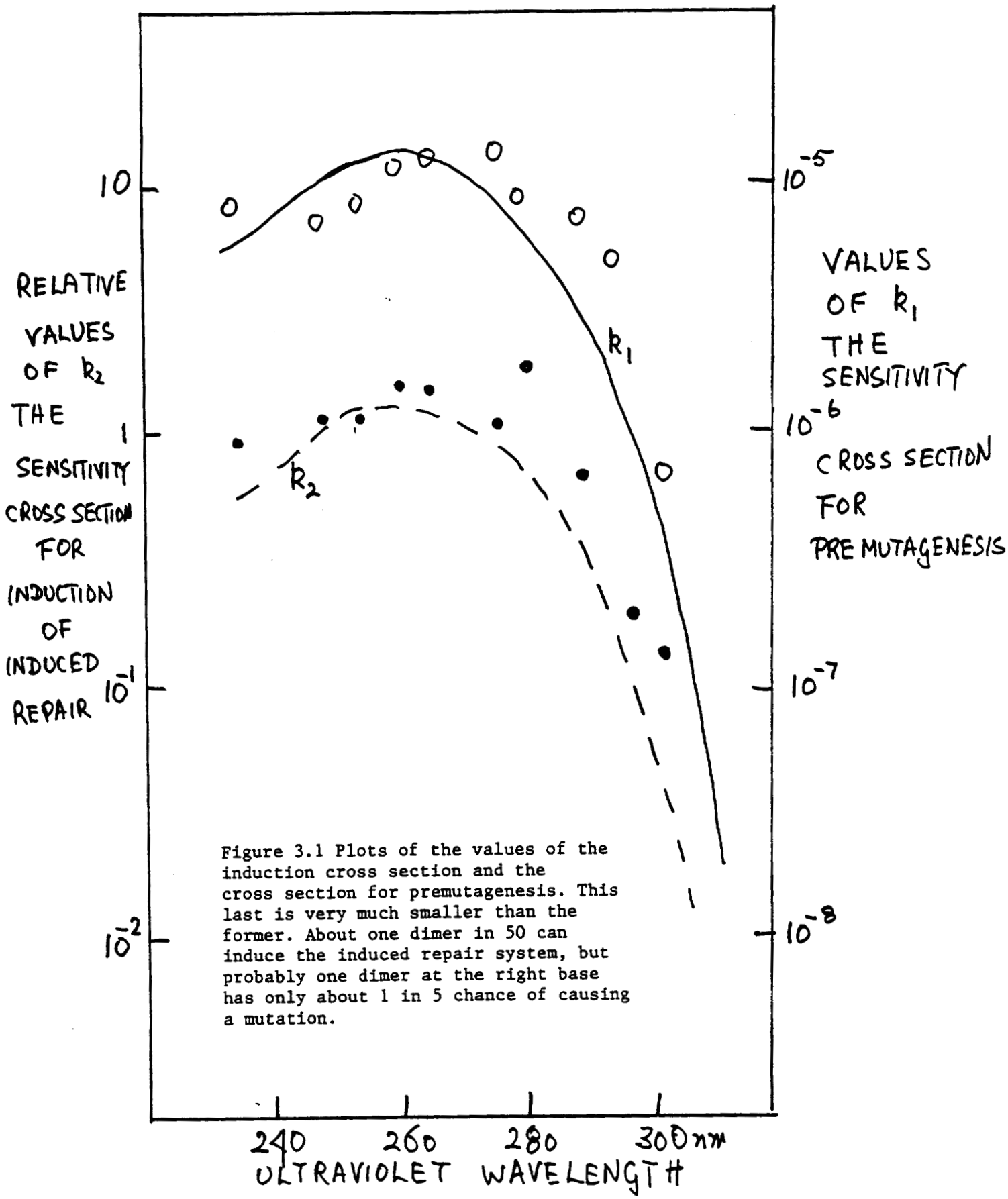


Figure 3.1 Plots of the values of the induction cross section and the cross section for premutagenesis. This last is very much smaller than the former. About one dimer in 50 can induce the induced repair system, but probably one dimer at the right base has only about 1 in 5 chance of causing a mutation.

1040000

cross section for the premutagenic lesion is very much less. In this case the probability of a dimer causing a mutation with the full mutagenic apparatus in effect is only about 10^{-7}. Since there are approximately 2×10^{6} base pairs per E.coli genome this suggests that only one in five of the dimers at the right location result in a mutation. This is not out of line with the idea that some abnormality in the functioning of the DNA repair apparatus is causing the faulty insertions. Four out of five could easily be right. It does, however, point up the relatively high probability of error, far beyond what the normal replicating machinery could be permitted.

Two other aspects of UV mutagenesis have been given some consideration in our work. We spent some time examining a suggestion that in strain WU3610 - 89 the very low dose range could give linear mutagenesis. This can be understood on the induced repair hypothesis by supposing that spontaneous induction has occurred in some cells and these mutate without the need to induce the induced repair system. We have not found such a region and for this strain it will require very elaborate plating to be sure that such mutagenesis shows above the "noise". On the other hand, in strain AB1886, observing histidine revertants we have moderate evidence that this linear with dose mutagenesis is taking place at low doses. We can not readily ascribe the relatively large yield to spontaneous induction. This needs more study. Sargentini and Smith report something of the same. The second aspect is the considerable increase in UV mutagenesis at high doses. Since here the surviving fraction of the cells is low we have had to work out a method of deducting the background correctly. This was described in last years report and has been applied in further work. This increase is real and not yet explained. We are in a position to work further on it. Preliminary work suggests that it is not equally present at all wavelengths, being harder to see at longer wavelengths.

APPENDIX 4

STUDIES OF WEIGLE-REACTIVATION FOR UV AND IONIZING RADIATION DAMAGED LAMBDA PHAGE

INTRODUCTION

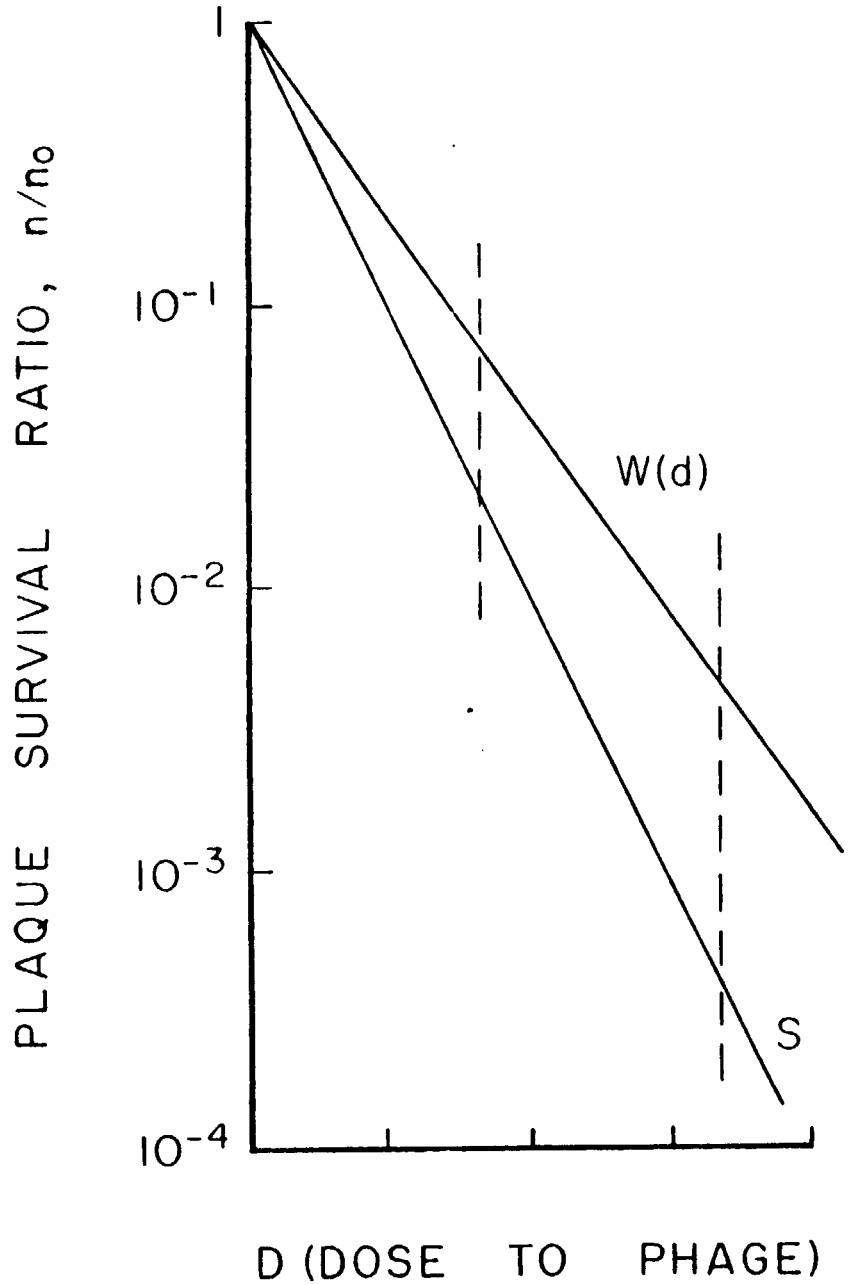
One of the earliest phenomena now associated with induced repair was discovered by Weigle and Ethel Tessman (1, 2). They found that UV damaged λ phage and T1 phage, respectively, were to some extent reactivated if they were adsorbed to irradiated host cells. The amount of reactivation depended on the amount of inactivation of the phage.

We have worked on this phenomenon with the intent of seeing if the dose-response for the development of Weigle-reactivation (or W-reactivation) after ultraviolet light matched that of other phenomena associated with induced repair. We have found that it does and this has been reported in a preliminary publication (3). In the course of further work we have also found that W-reactivation is shown for phage damaged by ionizing radiation. The degree of reactivation is not as great, but it is appreciable and in uvr$^-$ cells may actually be greater than the reactivation of UV damaged phage. This work was reported at the International Meeting of Radiation Research in Tokyo this year and a preliminary draft of the paper is what constitutes this appendix.

MATERIALS AND METHODS

Phage preparation

Since λ preparations are somewhat unstable (though we have recently found that they can be stabilized with low concentrations of gelatin), our procedure was to prepare a phage inductate on the day of the experiment. 2 to 3 x 10^8 cells of AB1157 λ were given an inducing exposure of about 100J/m^2 254nm UV. The cells were then incubated for two hours, giving a clear inductate of 3 to 4 plaque forming units per ml.



$n/n_0 = e^{-SD}$, usual survival ratio

$N/n_0 = e^{-W(d)D}$, reactivated by dose d to cells

$N/n = e^{[S-W(d)]D}$

$$\frac{\ln(N/n)}{\ln(n_0/n)} = \frac{[S-W(d)]D}{SD}$$

$$= 1 - W(d)/S$$

THE W-REACTIVATION FACTOR

$$\frac{S-W(d)}{S}$$

Figure 1. The basis for the definition of the W-reactivation factor.

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UV λ preparation

The inductate was exposed to about 3,500 J/m 254nm UV. The plaque survival was around 10^{-5}, not too accurately predictable.

Ionizing radiation damaged λ preparation

The inductate was clarified by low speed centrifugation, followed by a clarification of the supernatant at high speed. The phage pellet was resuspemded in 1/5 volume of adsorption buffer or 5x concentration nutrient broth. For stress on indirect action 50kvp X-rays were used on the buffer suspension of the phage. For direct action stress the same method was used, except that the phage were in 5x nutrient broth. Also preparations were dried out of nutrient broth on mylar foils and irradiated *in vacuo* with 1 Mev electrons. The damaged phage were then resuspended in nutrient broth.

Analysis of W-reactivation data

Figure 1 shows the basis for defining the W-reactivation factor. It is supposed that the plaque survival ratio obeys an exponential relation to the dose. This is being tested rather than assumed in work done currently. With this assumption the sensitivity factor S for survival on unirradiated host cells can be defined in relation to D the dose to the phage. When the host cells have received a dose d, there is a new relation in which we have used W(d) as the sensitivity factor, written in this way to show that it is a function of the dose to the cells and not a fixed property of the phage and the kind of inactivating radiation. It is then very simple to write the ratio of the two slopes on a semi-logarithmic plot as 1 - W(d)/S and this or its equivalent, $\frac{S - W(d)}{S}$ is what we use to measure the effectiveness of W-reactivation. This factor is independent of the dose given to the phage, whereas the ratio at any given dose, read directly off the two graphs increases steadily as one goes down the line. We thus use

$$\frac{\log (N/n)}{\log (no/n)} = 1 - \frac{W(d)}{S} \text{ or } \frac{S - W(d)}{S}$$

as our measure of W-reactivation.

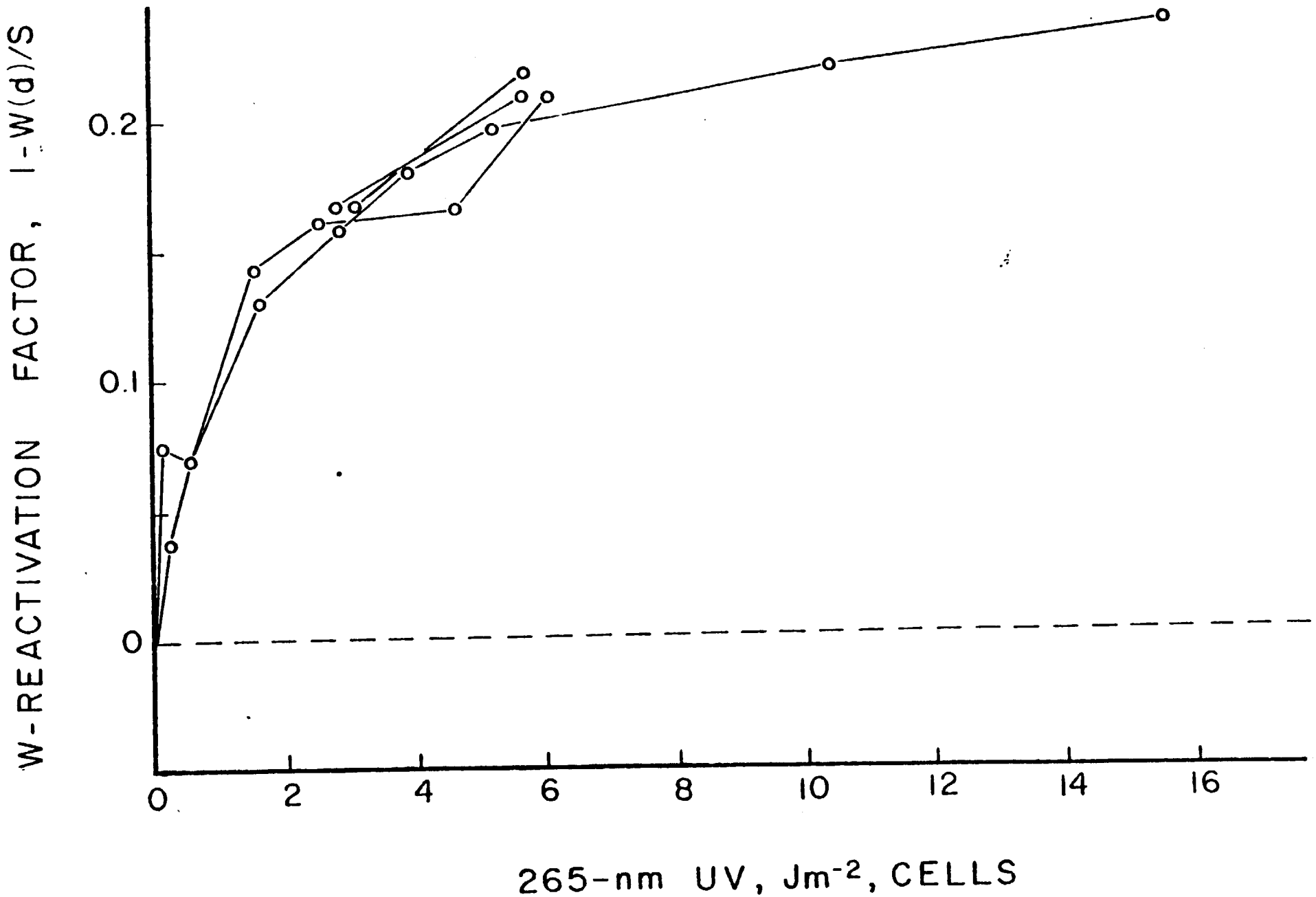

Figure 2. The observation of dose-response for the W-reactivation factor. UV damaged phage.

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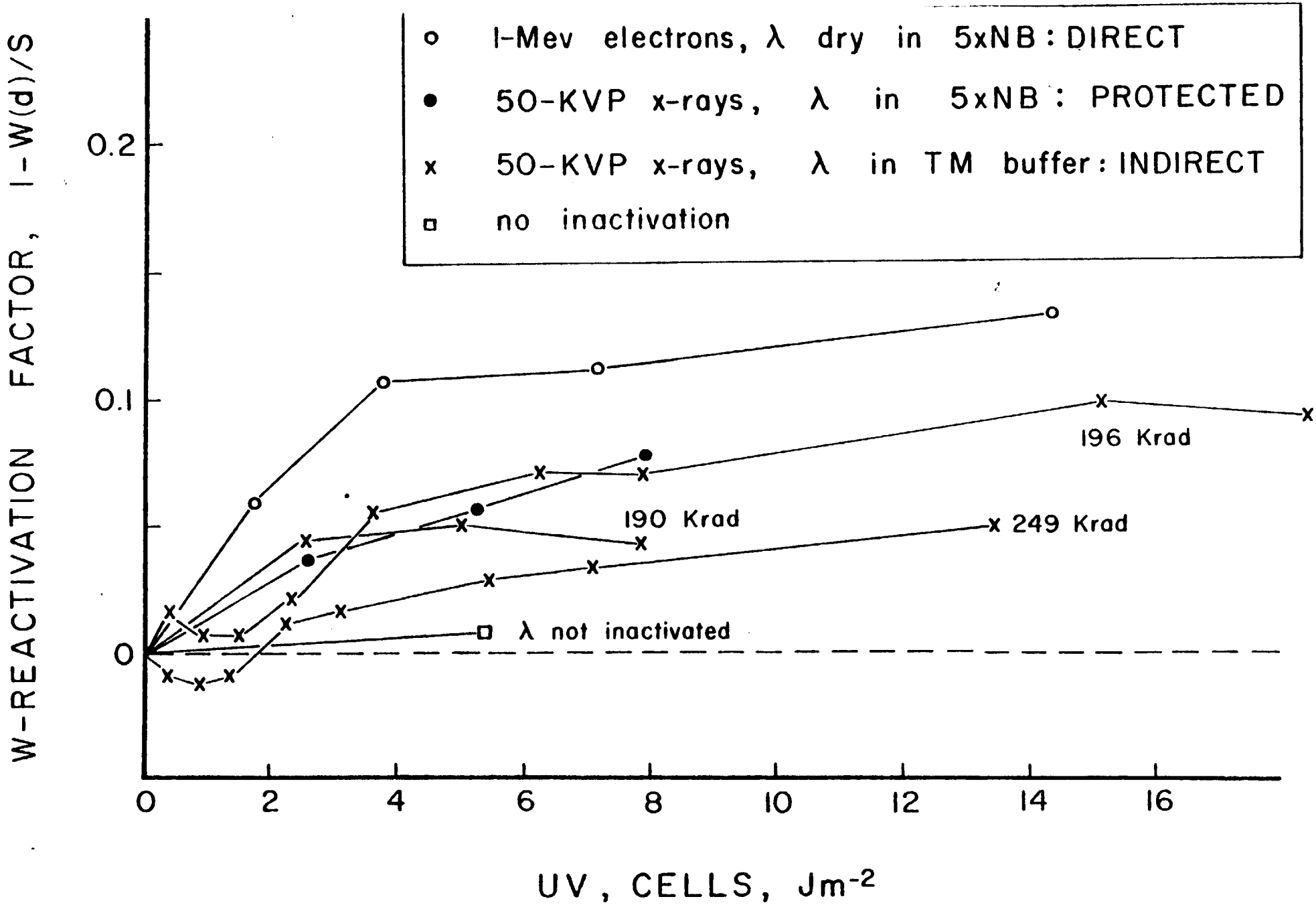

Figure 3. The dose-response for the W-reactivation factor for three modalities of ionizing radiation damage. The most reactivation is seen for direct action under anoxic conditions.

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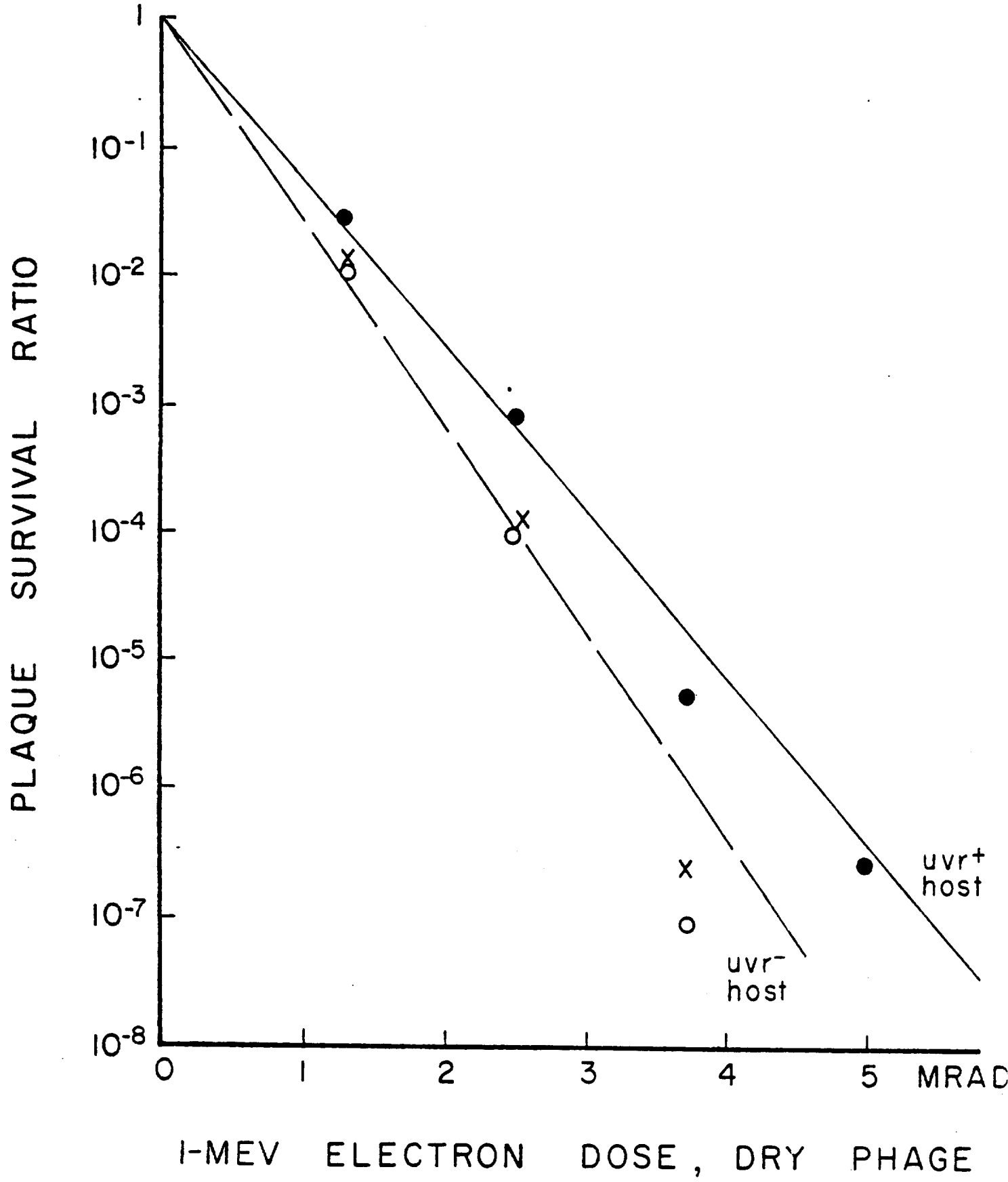

Figure 4. The difference in survival for λ phage inactivated by fast electrons in vacuum as between uvr+ and uvr- hosts. There is a component of recovery in the uvr+ case which is less in the uvr- case.

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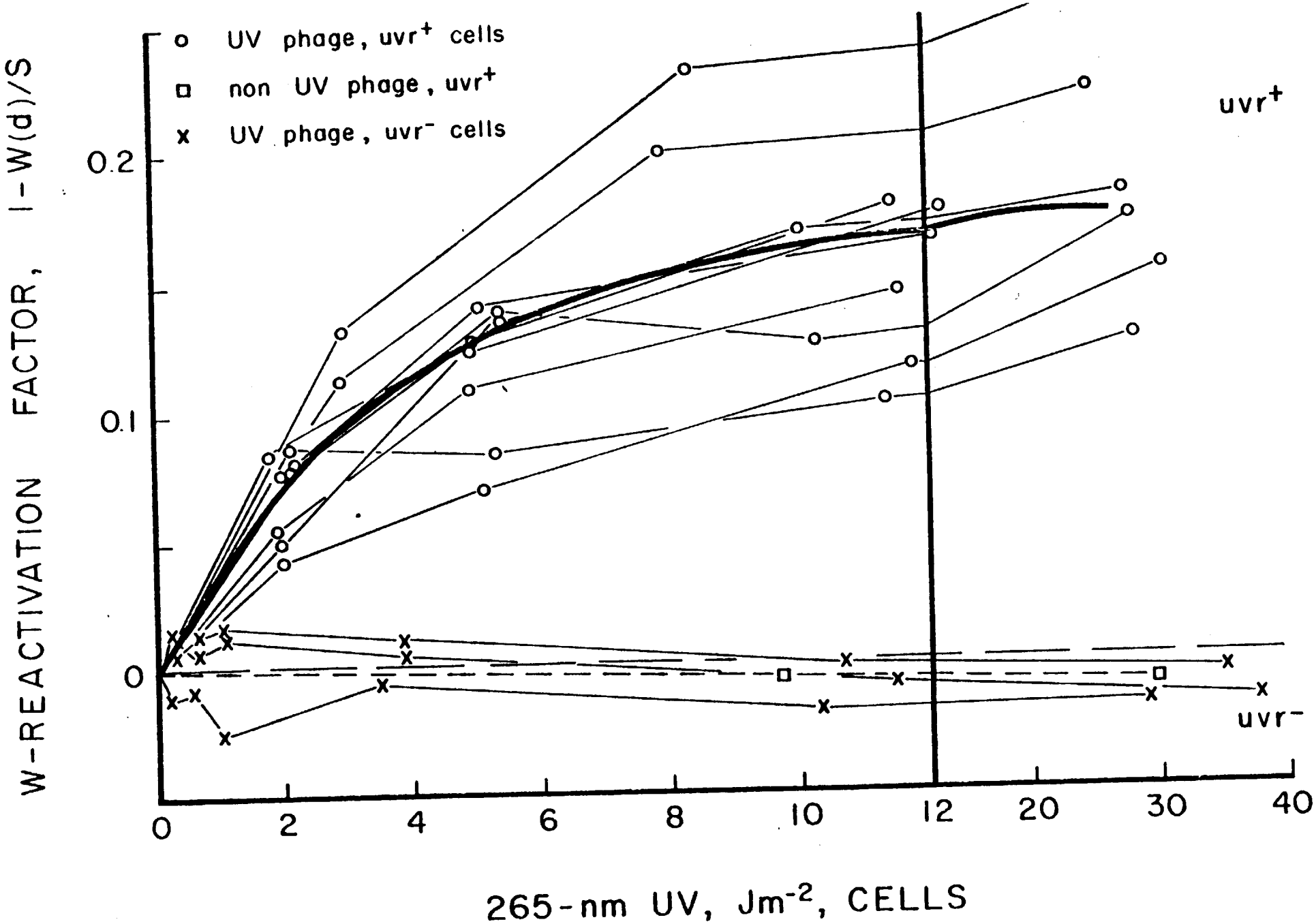

Figure 5. A diagram showing the dose-response data for a considerable number of trials. The thick line is a $1 - e^{-kD}$ relation, where D is dose. The observation of W-reactivation is always seen for uvr+ cells, but not for uvr−.

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RESULTS

Figure 2 shows the behavior of the W-reactivation factor for three cultures, using 265nm light to irradiate the cells. These observations were made with strain AB1157. It can be seen that the induction of W-reactivation follows a $1 - e^{-kD}$ type of relation, where D is dose. These three experiments were taken closely together in time. There is more spread in most of the data, as will be seen later. Probably there are factors about cultures which affect induction which we have not yer understood.

Figure 3 shows the same W-reactivation factor plot for three modalities of ionizing radiation damage. The one showing the best W-reactivation is the case of irradiation dry in vacuum with 1 Mev electrons. Indirect inactivation is on the whole less susceptible to W-reactivation, but there is still some effect. All these data were taken with strain AB1157.

Turning to work with the uvrA$^-$ strain AB1886, there is one observation of interest which is shown in Figure 4. This is that the inactivation of λ phage by fast electrons shows differently if plated on a uvr$^-$ host, an observation which parallels similar findings on T1 (4). Figure 5 shows a diagram of essentially all the experiments we have made on UV inactivated phage, with the W-reactivation factor plotted for both the uvr$^+$ and uvr$^-$ cases. We can not see any W-reactivation in the uvr$^-$ case. On the other hand, in one recently performed experiment, W-reactivation of damage due to fast electrons was observed in the uvr$^-$ case, indicating that the system for reactivation is active in the cells, though apparently silent for UV inactivated phage.

We have some data on mutagenesis to give clear plaques. This is shown in Figure 6. The data are quite sparse. They indicate that there is some W-mutagenesis seen in all cases of inactivation, but that it reaches a low maximum for ionizing radiation, while increasing for UV. The cells were AB1157 for these experiments.

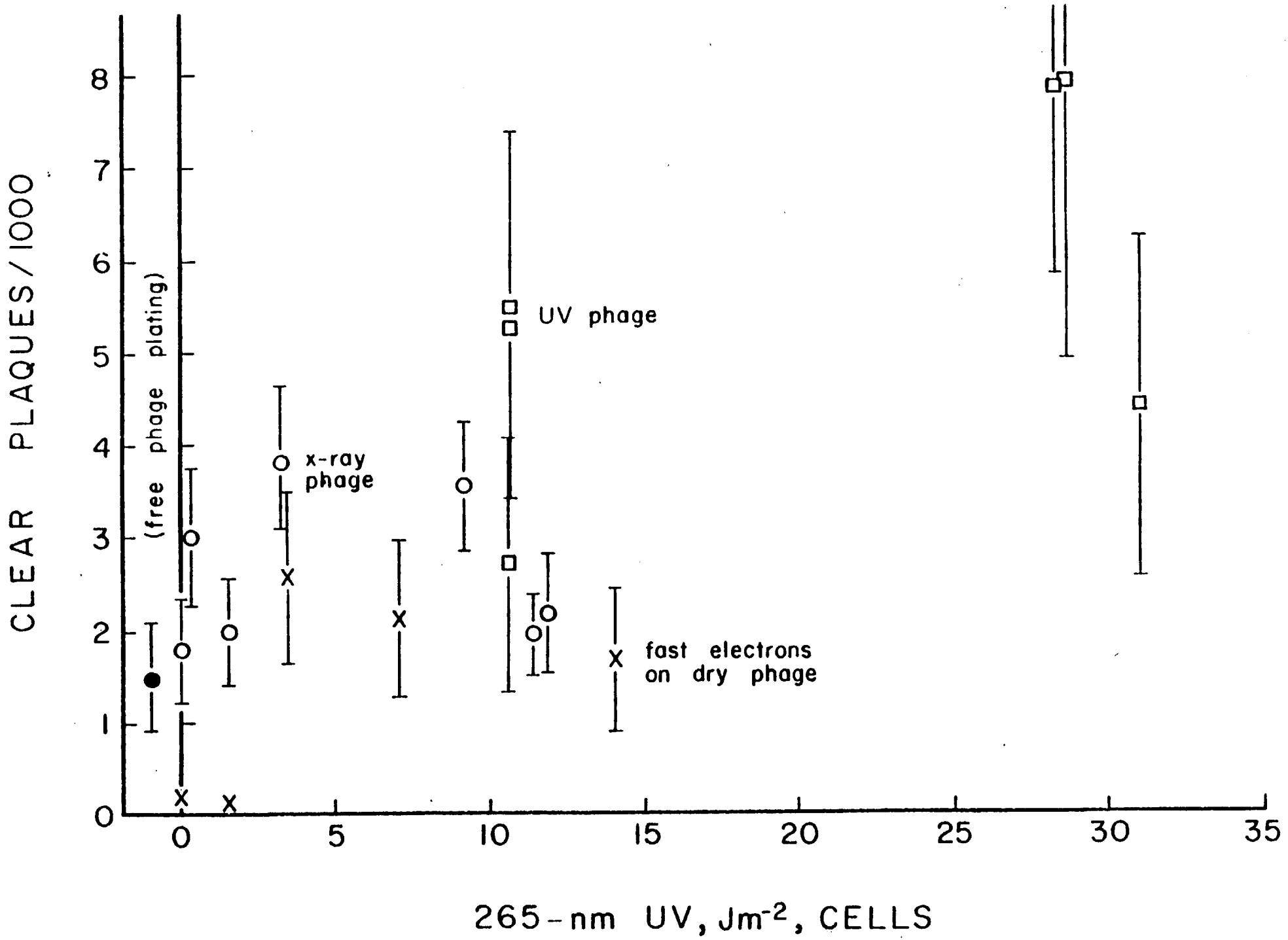

Figure 6. The observations of clear plaques due to a mutation in the cI region of the phage. These are clearly seen for all kinds of damage to the phage but show most definitely at high doses for UV damage.

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DISCUSSION

If we take the mean line, fitted to the $1 - e^{-kD}$ expression from Figure 5 then it is possible to see whether there is agreement between the dose response for W-reactivation for AB1157 and for other phenomena associated with induced repair. Some of this has been reported briefly elsewhere (3). The comparative induction lines are shown in Figure 7, from which it can be seen that W-reactivation follows closely the dose-response curve for the induction of protein X, the recA gene product, of septum inhibition, from which it is indistinguishable, of inhibition of postradiation DNA degradation, but it markedly deviates from the dose-response relation for the induction of λ. This is very sigmoidal in character and rises at higher doses. There is also some disagreement with the dose-response for induced radioresistance. We can conclude that the phenomena for induced repair do go together, with the exceptions we note. Probably the induction of λ requires a second UV generated process, as suggested by Marcovitch (4). The deviation for induced radioresistance may be determined by the different end point in this case.

The data shown in Figure 5 can be used to assess the degree to which we have failed to observe W-reactivation of UV damaged phage in AB1886 ($uvrA^-$). Others have observed that the W-reactivation factor is much less (5), but we are the only group to report essentially none. One difference between our work and some others is the plating indicator bacteria we use. It is possible that the use of fully repair deficient plating bacteria we use. It is possible that the use of fully repair deficient plating bacteria might show some difference. What intrigues us is the very recent (and not yet repeated) observation that we see normal W-reactivation of fast electron damaged phage in uvr^- cells.

Our observation that W-reactivation of λ phage damaged by ionizing radiation can occur to an easily measurable degree suggests that the repair system does not only operate on dimers. One very likely explanation is that the cells which have been ultraviolet irradiated contain the inducible inhibitor of post radiation DNA degradation and that this is preventing the degradation of the damaged phage to

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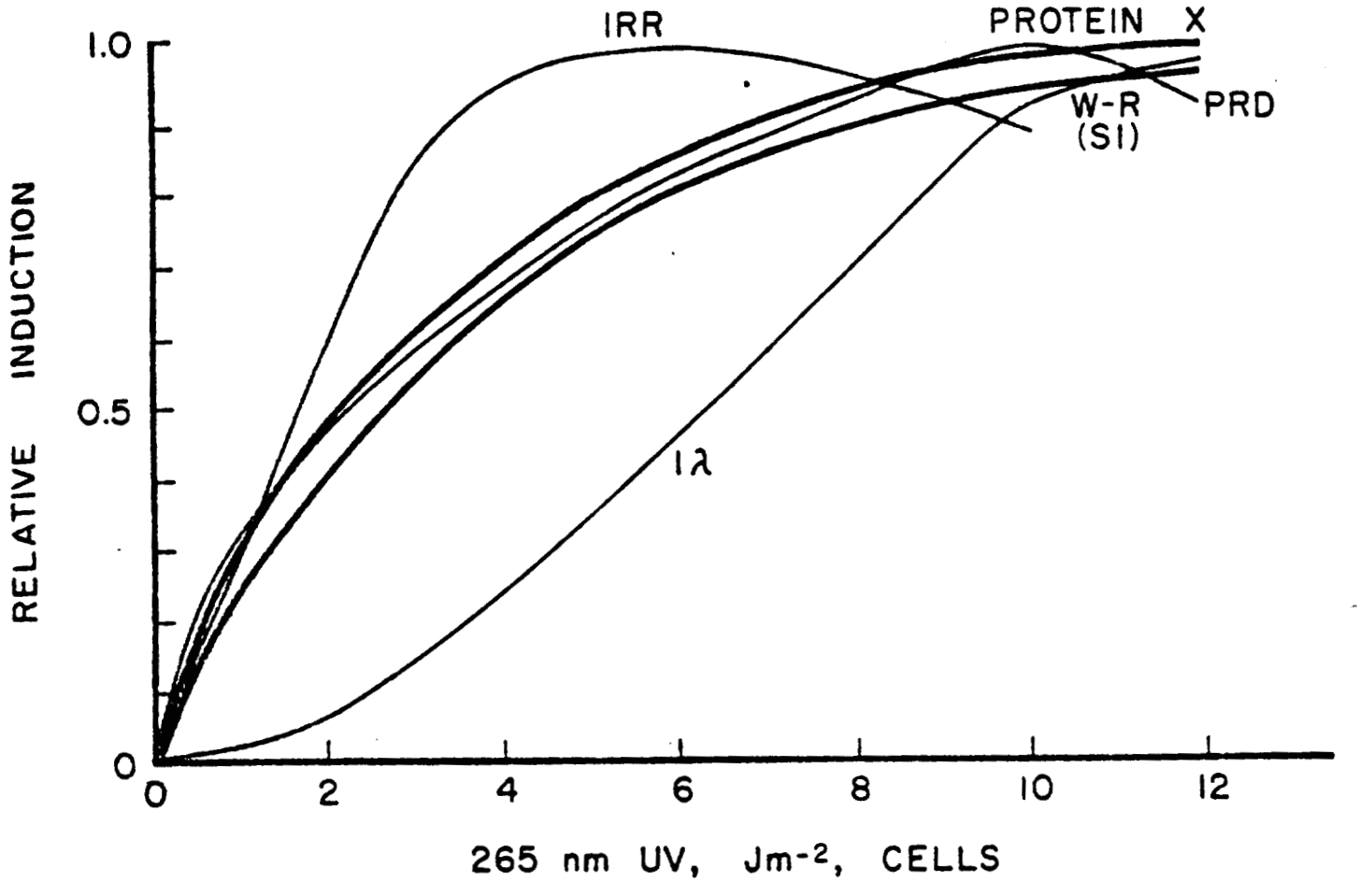

Figure 7. Comparative dose-response curves for the UV induction of six phenomena linked to induced repair. Four of these: protein X, septum inhibition, W-reactivation and inducible inhibition go together very well. The induction of λ does not. The observation of induced radioresistance is not in full agreement but occurs at lower doses and peaks early.

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the extent that there is now more survival. Such survivors might well contain more mutants, as observed. This idea can be tested by seeing whether there is W-reactivation on strain AB2470 (recB$^-$) which has inactive exonuclease V and would not appreciably act to degrade DNA which has been damaged by ionizing radiation. This is being planned.

ACKNOWLEDGEMENTS

We wish to acknowledge the help of Mr. Eric Bronner in many of the phage assays. We also acknowledge support from Contract No. EY-76-S-05-3631 with the U.S.Department of Energy.

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APPENDIX 5

THE DEVELOPMENT OF THE recA GENE PRODUCT: DOSE RESPONSE AND TIME COURSE RELATIONS FOR UV AND X-RAY INDUCTION IN W.T., recB, recF AND recBrecF STRAINS OF E.coli.

INTRODUCTION

The observation by Rothman and Clark (1) that the double mutant recBrecF is strikingly similar in sensitivity to the recA$^-$ mutant suggests that these two mutants involve loss of function of two processes which together make up the whole attribute of the recA$^+$ gene. The recB mutation involves the loss of function of exonuclease V, a multiple function DNA degrading enzyme. The recF mutation is thought to involve an endonuclease. In our laboratory we are making careful studies of the dose-response relations for the induction by UV and X-rays of phenomena which result from the derepression of the recA gene. It seemed to be important to study the dose-response relations for the recB and recF mutants to see whether they are different and, if so whether the difference can be related to the observation of Rothman and Clark. While doing this we have also found that the time course of the development of the recA protein, which we shall refer to as "precA", which is synonymous with "protein X", is different in these mutant strains. Our findings lead us to suggest that there are two ways in which recA can become derepressed and that both are probably operating in the wild type.

MATERIALS AND METHODS

Bacterial Strains

The strains we used were AB1157, a K12 strain which is wild type in the radiation sensitive mutants. AB2470 (recB), JC9238 (recF) and JC3881 (recB recF)

Observation of precA

The now familiar band of "protein X" was observed by gel electrophoresis using an 8 to 16% or 8 to 20% gradient gel. Two methods of observing quantitative yields of precA were employed. The first involved using radioactive labeling with ^{35}S,

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cutting out the band which has been stained with Coomassie blue, dissolving the gel and band in 30% H_2O_2 and counting the solution in a scintillation counter. Comparison was made with the remainder of the gel, also dissolved in the same way. The second, primarily used for the time course experiments involved cutting out the band and exposing it to destaining solution at 54°C for an hour or two, or at room temperature overnight, and then reading the optical density of the solution in a spectrophotometer. Again, comparison was made with the rest of the gel.

Irradiation procedure

For UV, monochromatic light at 265nm was used. This was delivered by a double prism monochromator. The light source was a mercury arc at high pressure cooled by a rapid stream of air. For X-rays a Picker 50kvp machine, with a Machlett tube and 50mg/cm^2 Aluminum filtration was used. Doses were measured by calibration with a ferrous sulfate dosimeter.

RESULTS

Dose response relations

Figure 1 shows the amount of precA as a fraction of the newly synthesized protein (i.e. radioactive assay) for different doses of UV. The cells were allowed to incubate for 60 minutes after the inducing dose. It is quite clear that the recF mutant develops full yield at quite low doses, while the recB strain gives slightly more yield but requires considerable exposure to UV to generate the maximum. The summation of the two lines is somewhat like the response of the wild type. The double mutant, JC3881 is not wholly void of yield so the two mutant strains do not entirely exhaust the ability of the cell to cause generation of precA. If the incubation time is shorter the yield curves look as in Figure 2. Significantly the yield of the recF mutant is lower and at this time the summation of the two mutant yields is very close to that of the wild type. This is significant, though it is doubtful if the rather remarkable agreement at this time is more than a little lucky.

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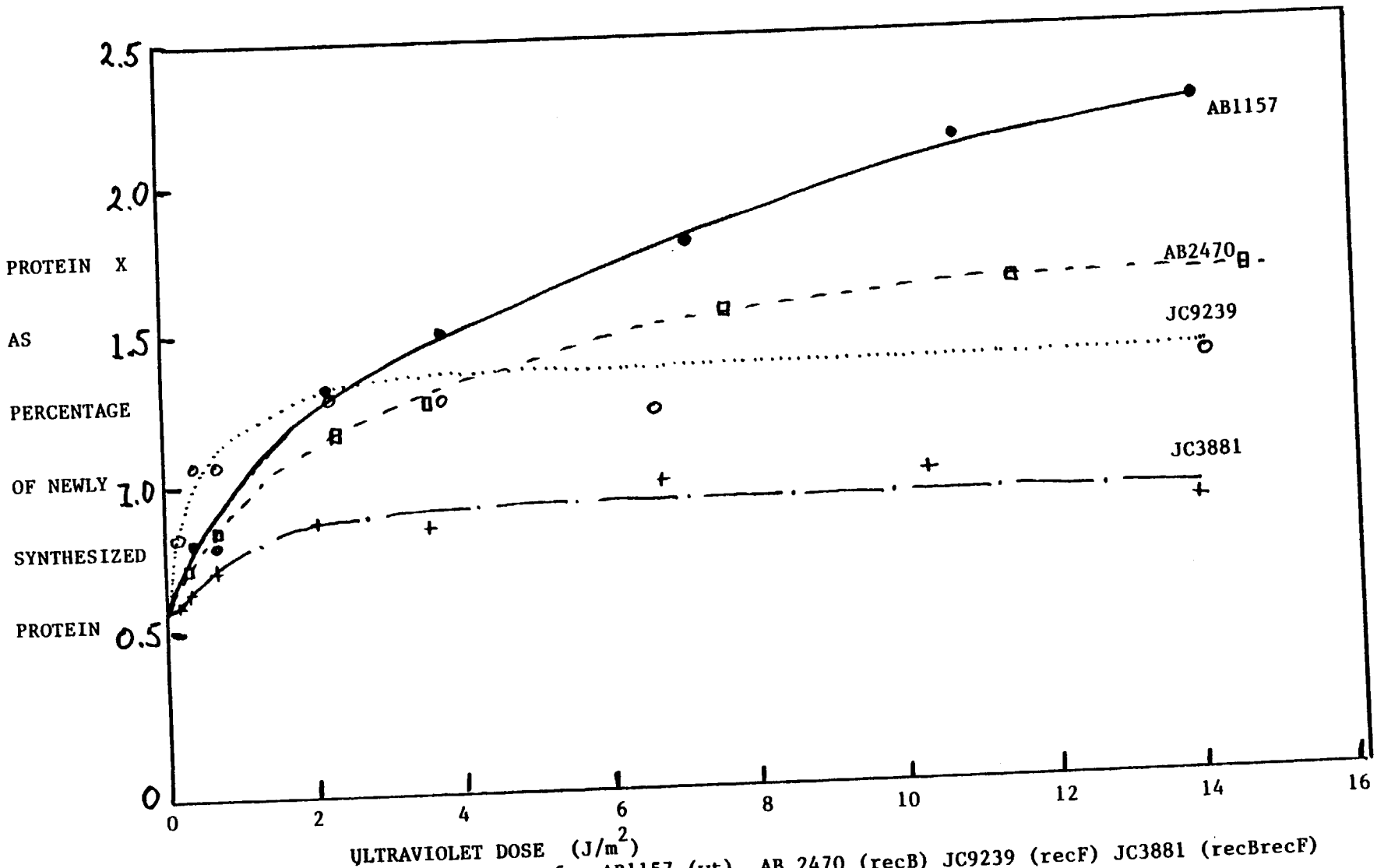

Figure 5.1 Dose response curves for AB1157 (wt), AB 2470 (recB) JC9239 (recF) JC3881 (recBrecF) Incubation time 60 minutes.

Figure 5.

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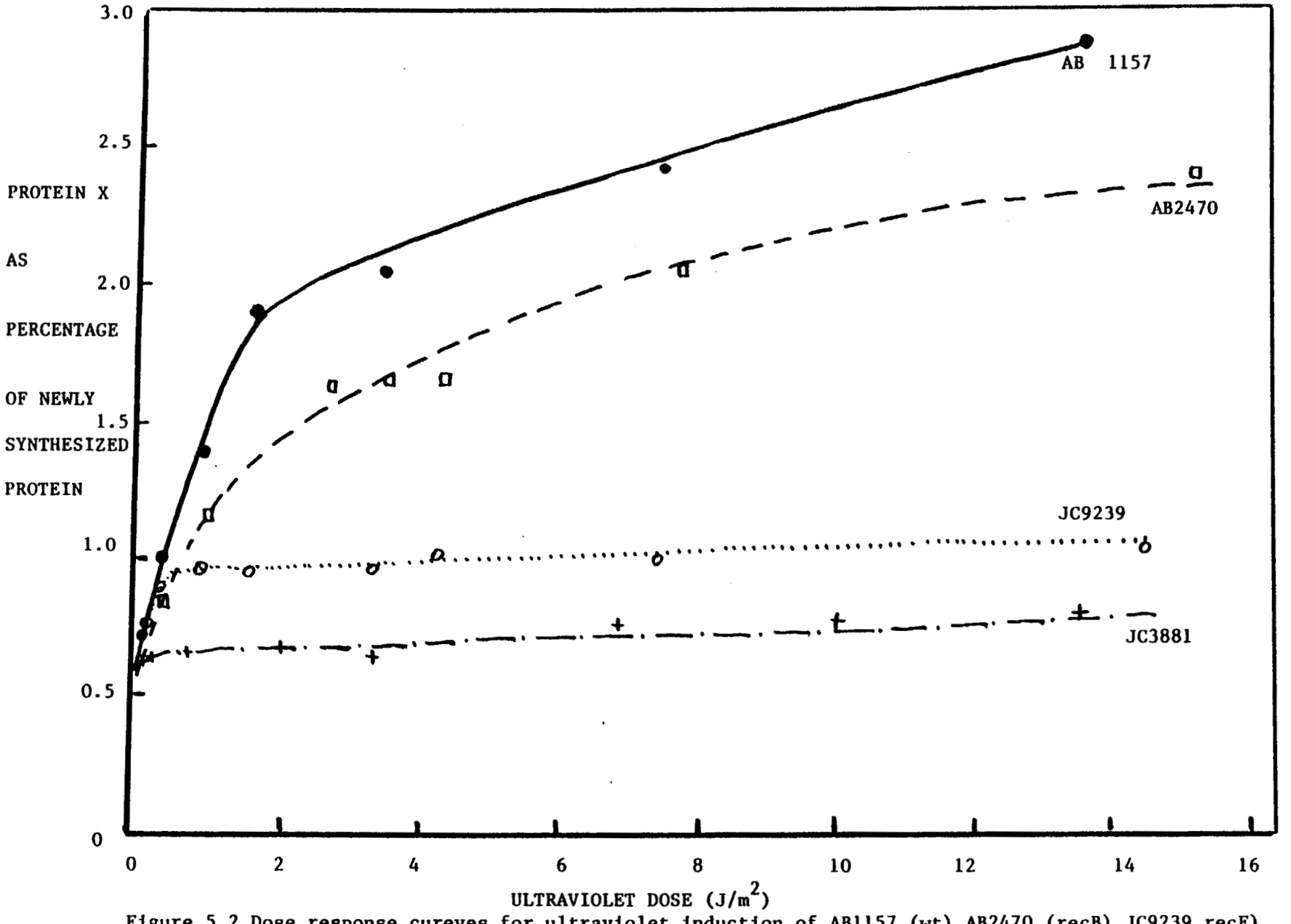

Figure 5.2 Dose response cureves for ultraviolet induction of AB1157 (wt) AB2470 (recB) JC9239 recF) and JC 3881 (recB recF). Incubation time 30 minutes. Note the lower yield of the recF strain.

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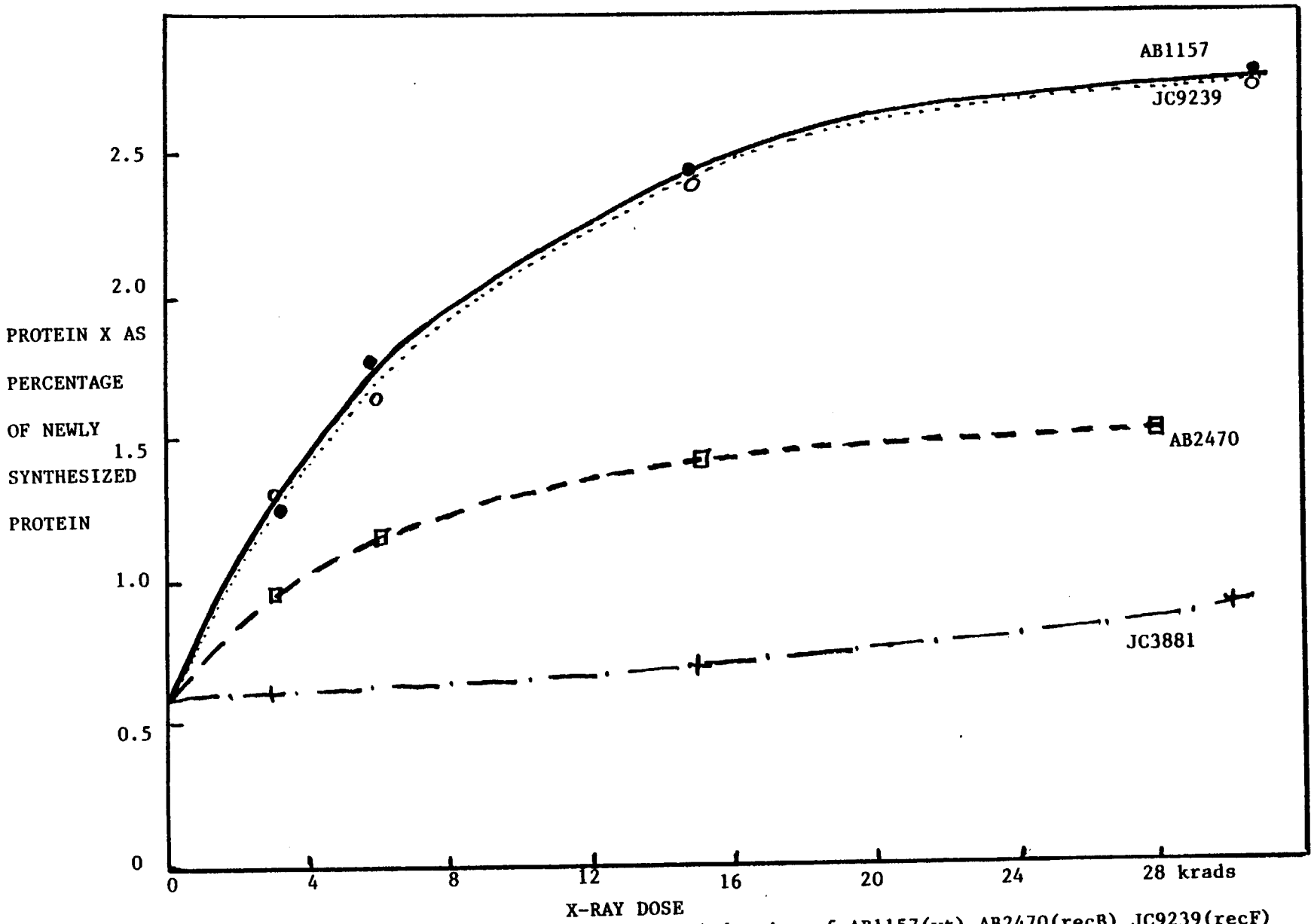

Figure 5.3 Dose response relations for X-ray induction of AB1157(wt) AB2470(recB) JC9239(recF) and JC 3881(recB recF). The wild type and recF strains are indistinguishable. recB is much loer.

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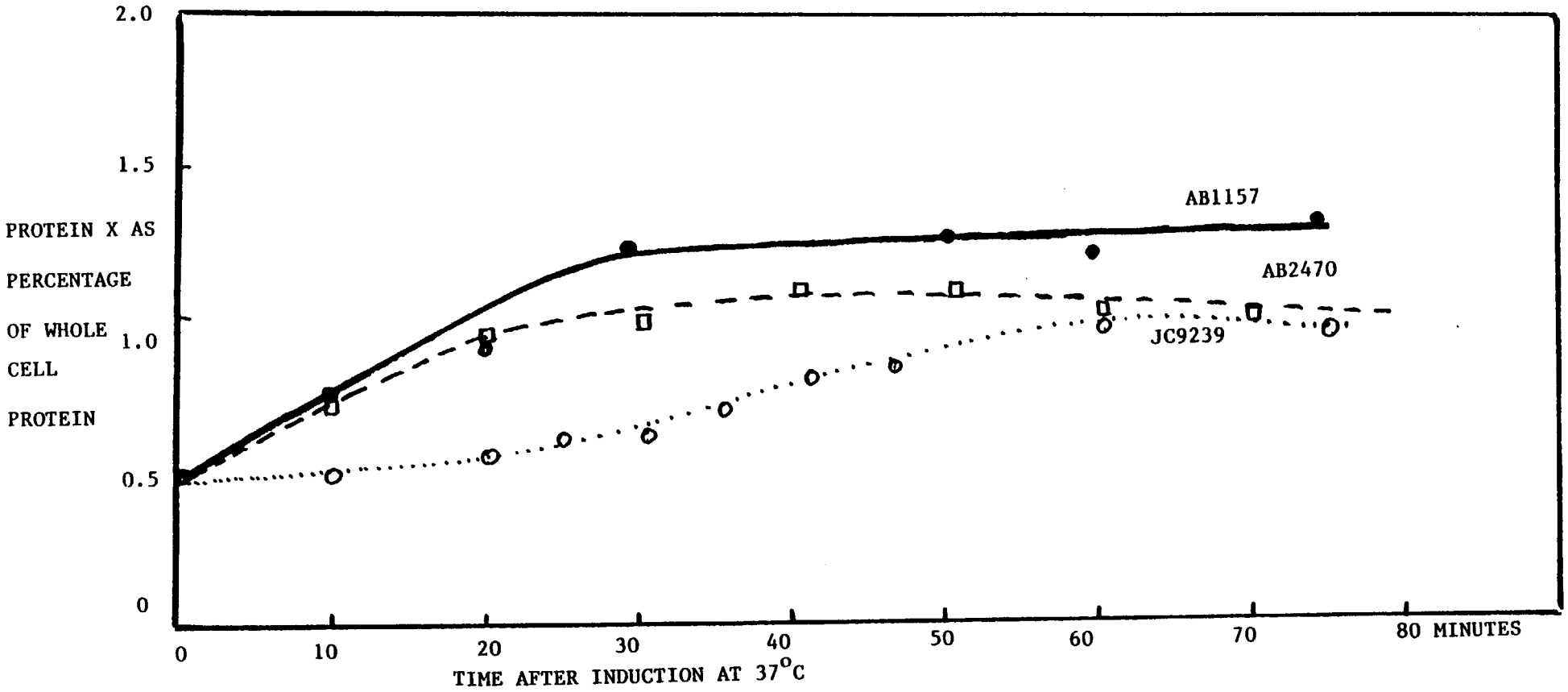

Figure 5.4 The time course of induction of AB1157(wt) AB2470 (recB) and JC 9239(recB recF) with $10J/m^2$ UV inducing dose for AB1157 and AB2470 and $2J/m^2$ for JC9239. Although this dose is about maximally effective for the recF strain, the recA gene product does not develop until after 20 minutes or so, in striking contrast to the other two strains.

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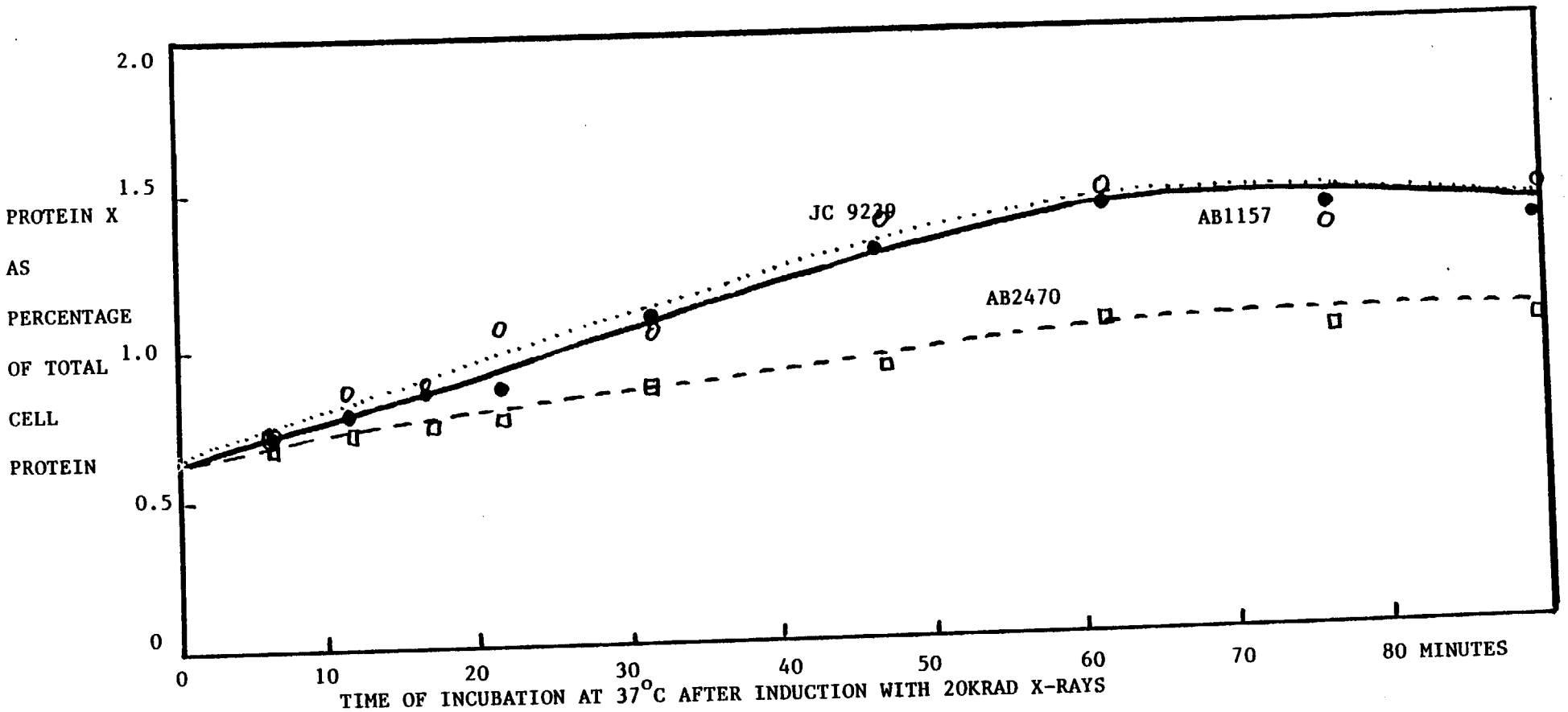

Figure 5.5 The time course of induction of strains AB1157 (wt) AB 2470 (recB) and JC 9239 (recF). For this mode of induction there is no time delay in the onset of the appearance of the recA gene product. The yield of the recB strain is always lower, probably reflecting the lack of breakdown products due to the absence of the action of exonuclease V.

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If X-rays are used as the inducing agent there is a very different pattern. In the first place the yield of the recF mutant almost matches that of the wild type. In the second place the recB mutant gives a much lower yield, whereas with UV the yield was greater than the recF case. There is almost no yield from the double mutant. Figure 3.

Time course relations

Figure 4 shows the time course of the development of precA after the doses shown of UV. Here the assay is by the colorimetric method on the strain and the percent is of the total cellular protein. The most significant observation is the considerable delay before any precA appears in the recF case. Almost the full yield of the recB strain has appeared before any precA develops from recF. Again, in a rough manner the summation of the two mutants produces the wild type curve. When induction is by X-rays, the results of Figure 5 are seen. Here there is no time delay, though all the induction takes longer and the maximum of induction is not attained for any mutant before 60 minutes.

DISCUSSION

If we take the case of X-ray induction first we can see the clear result that the mutant which lacks the degrading enzyme exonuclease V, namely recB, induces less over the whole dose range. Since there is considerable breakdown of DNA after X-radiation it is reasonable to suggest that the lack of this breakdown is responsible for the low yield. Thus we can conclude that there is an induction due to breakdown products and also one which is not so dependent on them.

Turning to the UV induction case, the generation of breakdown products after UV is about five to ten times less than for X-rays. Strain AB2470 is recB$^-$ and so generates relatively few breakdown products. Yet if the dose given is sufficient the induction of precA is at nearly normal amounts and the time course shows no initial delay. Some inducing agency is being developed by the enzymatic potency of the recF genome and this is evidently better at induction than the same machinery presented with X-ray damage. On the other hand, if the strain has exonuclease V

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fully active but lacks the recF machinery there will be degradation products and these can be the inducing agent. Such products will follow by enzymatic action even if there are only a few damage sites, for exonuclease V, once it begins degradation continues for some stretch, if not the whole genome. However, it does so relatively slowly. Therefore the recF$^-$ strain will be expected to produce precA with a time delay, but do so in reasonably good amount. This observed.

Because we see clear differences in the development behavior of these two mutant strains we suggest that there are two ways in which the final derepression of the recA gene can take place. We can suggest hypothetical processes, but at the moment have no evidence to give in proof of them.

ACKNOWLEDGEMENTS

We acknowledge support from the Zoology Department, Duke University, in the form of a Hargitt Fellowship for one of us (D.K.) and support from the U.S.Department of Energy Contract No. EY-76-S-05-3631.

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APPENDIX 6

COORDINATE INDUCTION IN recB and recF STRAINS OF E.coli.

INTRODUCTION

The observations noted in the previous account indicate that there are two modes of derepression of the recA gene and that these can be seen by studying the development of the recA gene product, which we refer to as precA. This difference in development suggests that other phenomena which result from the derepression of the recA gene be looked at, using the approach of induction with ultraviolet light, absolute dosimetry, and monochromatic light. This is more limited in the case of these two strains because the absence of the degrading enzyme exonuclease V in recB strains eliminates the observation of inducible inhibition of post radiation DNA degradation (IIPRD) and also, rather curiously, induced radioresistance (IRR). There remain the observation of septum inhibition and of the induction of λ. Moreover, the degree to which IIPRD and IRR show in recF strains is of some interest. Accordingly we studied these four phenomena in strain AB2470 which is recB$^-$ and JC9238 (recF). We also made some observations on the double mutant JC3881 (recBC recF). We find that the induction of these phenomena is reasonably coordinate in nature, with some slight surprises. The induction of λ proved to be readily observable in recF, in contrast to one report in the literature (1), and also in recB. The inducible inhibition of post radiation DNA degradation was readily observable in recF, but there were signs that it is readily saturated. The phenomenon of induced radioresistance shows in recF cells, but to a relatively low degree, not what we expected. An account of our observations follows.

MATERIALS AND METHODS

None of the techniques contain any novelty. Septum inhibition was observed by photographing cultures at 90 to 100 minutes after exposure to UV and scoring the percent of cells outside the normal length. It is fully described in Appendix 7.

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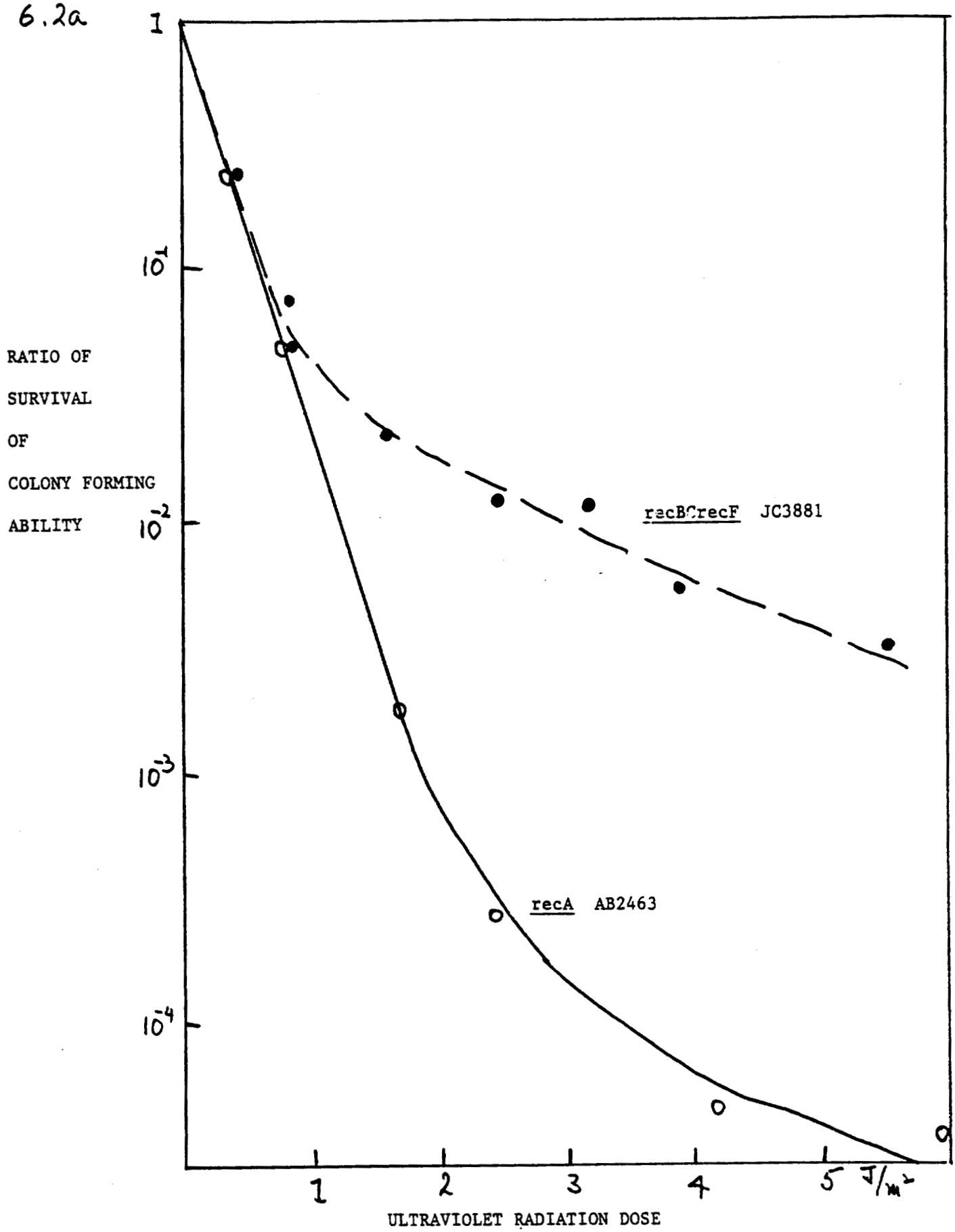

Figure 6.1 Survival curves for ultraviolet radiation of recA and recBrecF. While the two are different in the lower survival ratios, all but 10% are essentially of the same sensitivity.

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6.2b

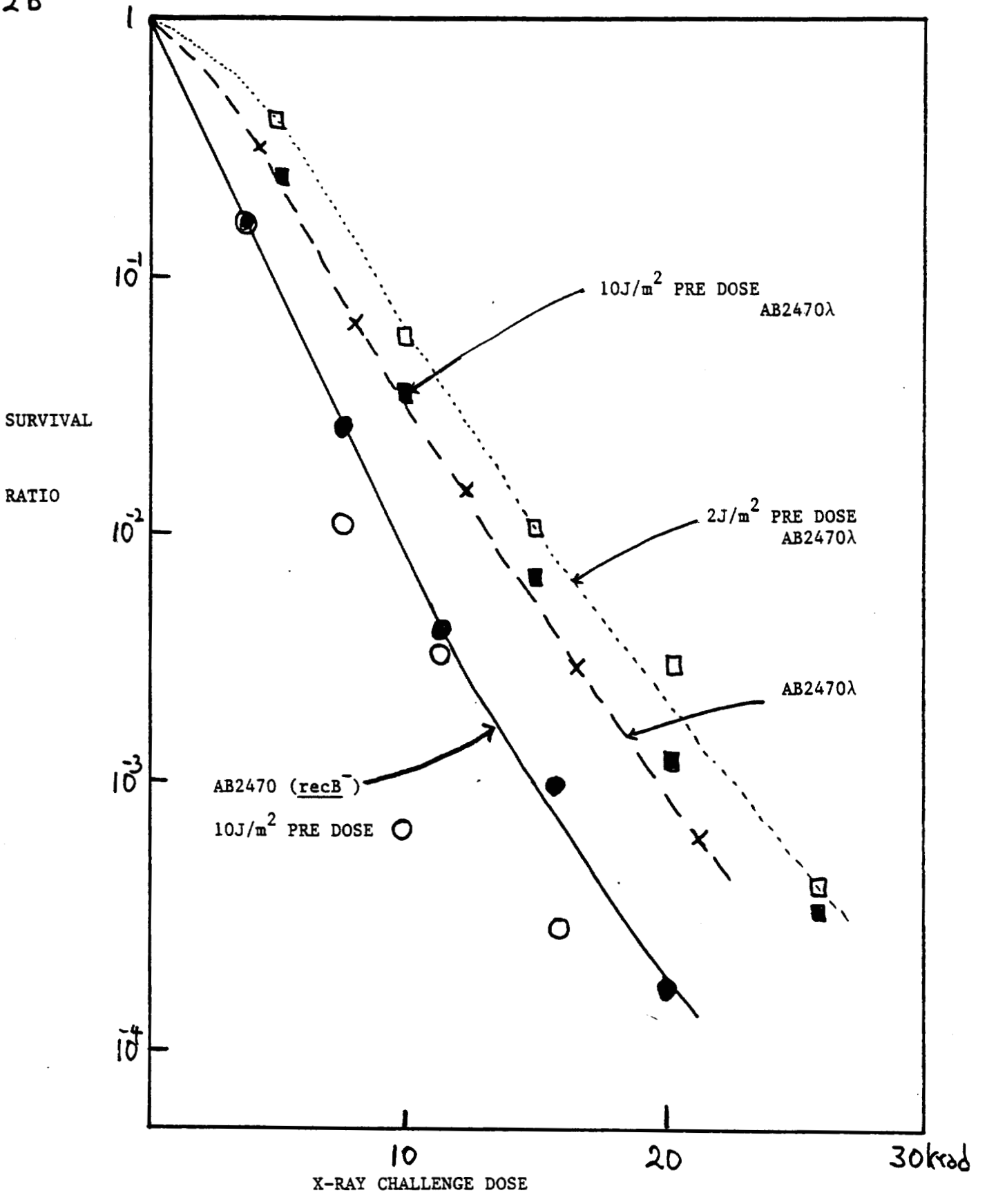

Figure 6.2 The lack of induced radioresistance in strain AB2470 (recB$^-$). The filled in circles and the open circles refer to the survival of cells of AB 2470 without and with a pre dose of UV. No increased resistance is seen. In the lysogen a small amount can beelicited, about at the limit of what can be claimed.

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6.2c

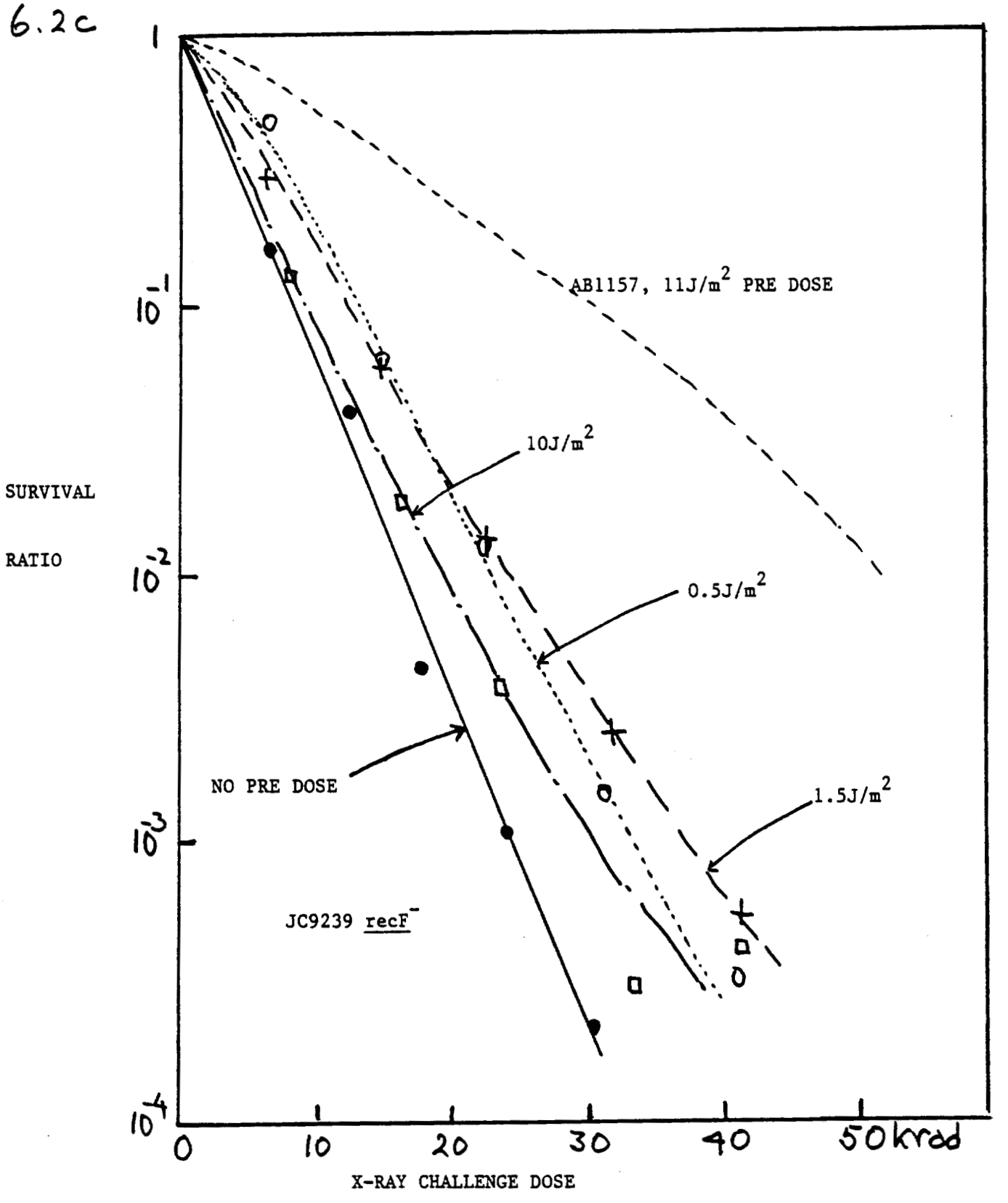

Figure 6.3 The observation of radioresistance in strain JC9239 recF$^-$. The cells which have received a pre-dose of UV are definitely more resistant, though not to anything like the extent seen in strain AB1157 which is essentially wild type. The doses to elicit radioresistance are quite small.

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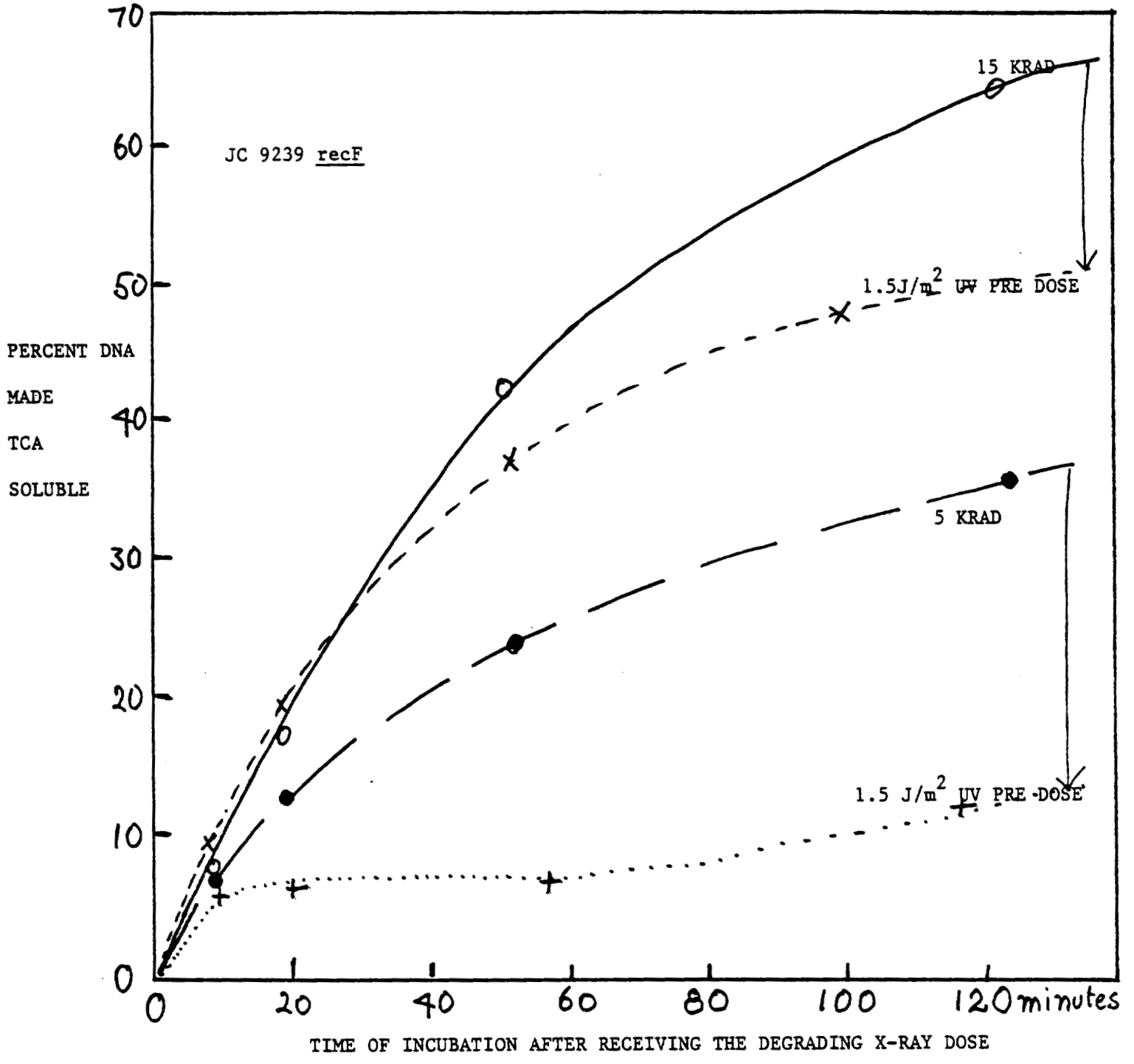

Figure 6.4 The inducible inhibition of post radiation DNA degradation in strain JC9239 (recF). Cells which have received a predose of $1.5 J/m^2$ UV dp not degrade their DNA after the X-ray exposure to as great an extent. The effect is more marked for low X-ray treatment, suggesting that the inhibiting action can be saturated by high doses of X-rays.

Inducible inhibition of post radiation DNA degradation was observed by labeling the cells with ^{3}H-thymidine prior to exposure to UV, incubating for 40 minutes, adding rifampin for 10 minutes and then exposing the cells to various doses of X-rays. The percent of solubilized DNA was then observed, by adding the cells to 5% trichloroacetic acid, waiting 30 minutes, centrifuging and counting the supernatant. The induction of λ was followed by observing the number of infectious centers at 40 minutes after UV and the number of free phage at 120 minutes after exposure. The irradiation techniques have been described elsewhere and appear in this report.

RESULTS

Figure 1 shows the loss of colony forming ability in the two strains JC3881 which is recBC recF and AB2463, which is recA. The two do not show identical sensitivity, but the difference is due to a resistant component, present to 3 in 10,000 in the recA$^-$ and about 1% in the recBC recF strain. Kazanis has shown that there is a small amount of precA developed in the recBC recF strain, while there is none in the recA strain. Thus there is some difference, but it affects only 1% of the cells.

Figure 2 shows the lack of induced radioresistance in the recB strain. There is absolutely no increased resistance following a 10J/m^2 predose of UV in the non-lysogen. In the lysogen, which it will be noted, is somewhat more resistant to X-rays, there is a barely detectable induced radioresistance. In Figure 3 we show the same kind of study on strain JC9239, recF. There is clearly radioresistance, shown only markedly at low doses, and being very much less than that seen in the wild type.

Previous studies reported in our progress reports by Kathy Keller (2) show that in the recB strain where there is very little post radiation DNA degradation at all, there is also no inhibition of what is there. Figure 4 shows data taken with the recF strain. There is clearly plentiful DNA degradation at X-ray doses which are relatively low. If 1.5 J/m^2 pre-dose of UV is given there is inhibition of degradation which shows much more clearly when the degrading dose of X-rays has been small. Thus the ability of the precA to bind to the DNA and prevent degrada-

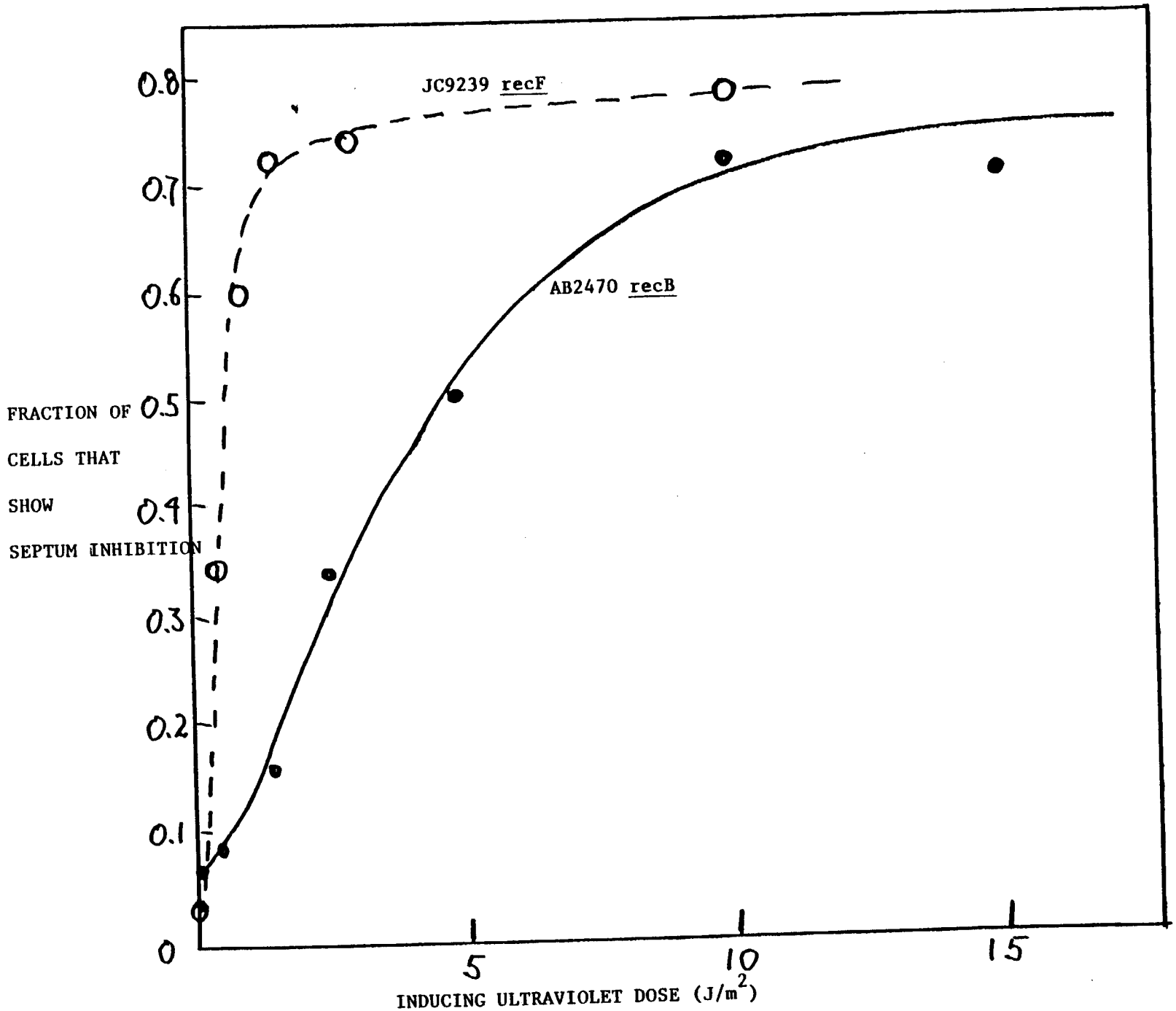

Figure 6.5 The induction of septum inhibition in recB and recF strains of cells. Both show the phenomenon, over differing dose ranges.

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6.3b

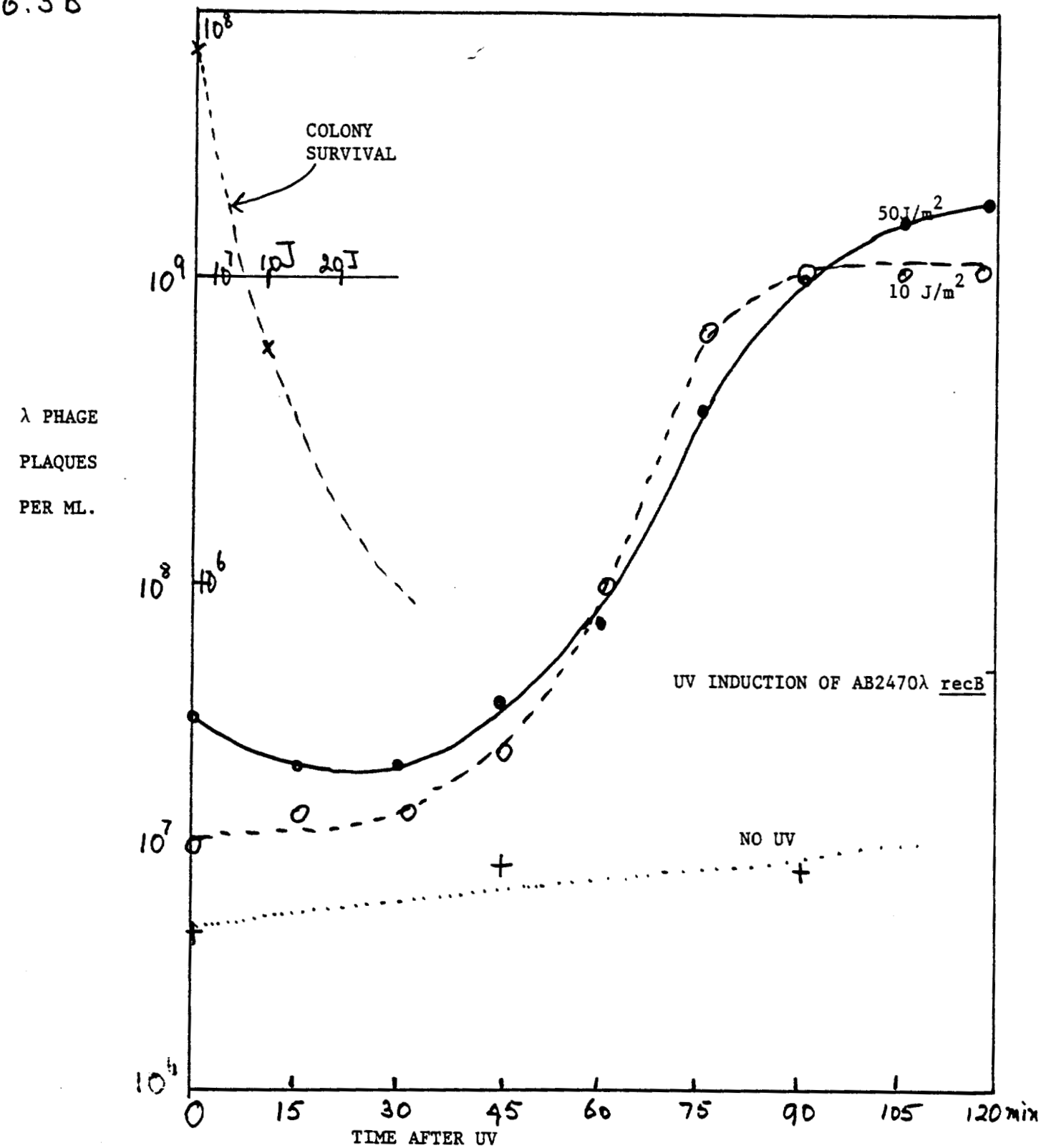

Figure 6.6 This illustrates the method used to observe the induction of phageλ. In this instance two doses have been given and the time course of the development of plaques has been followed. The cells were also plated for survival as shown in the upper left. The plaques observed at 40 minutes were considered to be due to infectious centers and at 120 minutes to free phage. The burst size is relatively large in the recB strain.

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6.3c

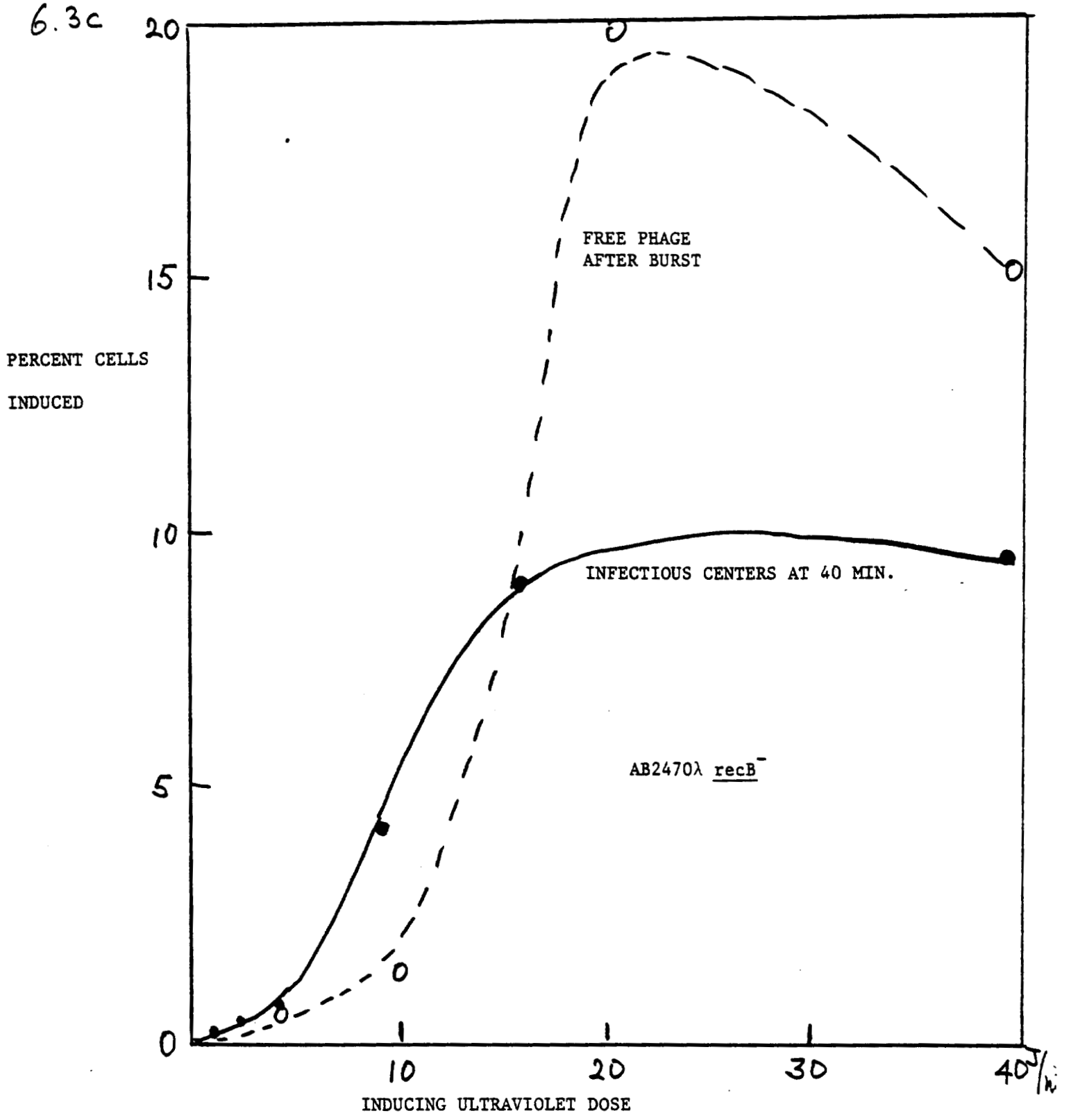

Figure 6.7 The dose response relation for the induction of phage λ in AB2470λ. Both methods show a sigmoidal induction curve, characteristic of the induction of λ. The two methods do not agree in detail because of the uncertainty of the plating efficiency of infectious centers on the one hand and of the burst size as dose changes on the other.

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6.3d

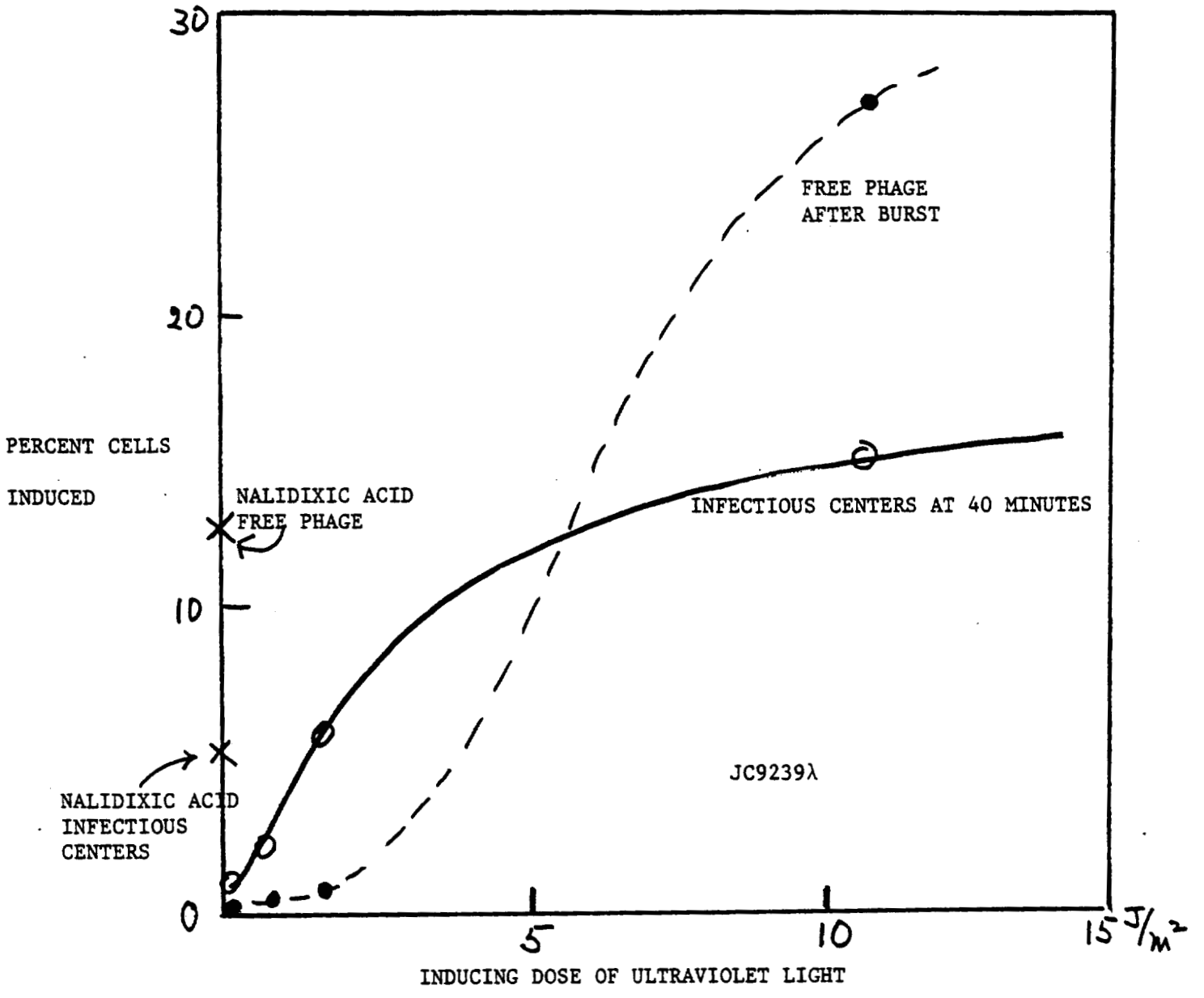

Figure 6.8 The induction of λ phage in strain JC9239 λ (recF⁻). Ultraviolet light is a potent inducer, being more efficient than nalidixic acid.The dose-response has a sigmoidal character.

tion is more than usually limited in this strain. We refer to this as saturation. It is normally seen to show at about 25krad dose of X-rays. Here it shows at more like 10. Nevertheless IIPRD can be seen.

Figure 5 shows the observation of septum inhibition in the two mutant strains. The percent of cells of length beyond the normal spread is plotted against the inducing dose. For the recF case there is a rapid increase in septum inhibition, rather matching the rapid increase in precA with dose. For the recB case there is also septum inhibition and this again occurs in the dose range which corresponds to the development of precA. Thus this phenomenon is clearly related to the induction of precA in both strains.

Figure 6 shows the method used to observe the induction of λ. The lysogen of AB2470 has been given either $10J/m^2$ or $50J/m^2$ of 265nm UV light and the plaque titer measured at various times after the inducing irradiation. At the same time the loss of colony forming ability is observed. The production of a burst of phage is clearly seen. Also it can be seen that for considerable UV doses there is a time of high sensitivity of the infectious centers which lose their plating efficiency to some extent. This can be seen in the data of others workers. For this reason we plate for infectious centers at 40 minutes, before the burst and at 120 minutes for the full burst. In the case of AB2470 λ, we observed large burst sizes, and indeed, this is an attractive strain to use as indicator phage. In Figure 7 we show the dose response curve for the induction of λ, derived either from observation of infectious centers or from free phage with an assumption of a burst size of 50. The uncertainty in the burst size with varying dose is a problem for free phage observations. The plating efficiency of the infectious centers is a problem for the infectious center observations. However, it can be seen that λ is induced and that the characteristic sigmoidal dose-response relation is seen. In Figure 8 we show observations on the recF strain. The lysogen is again quite clearly induced with inducing doses much less than those observed for the recB lysogen. On the ordinate are shown the yields for induction with nalidixic acid. Ultra-

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violet light is actually more efficient in this instance. Again the dose response relation is sigmoidal as we have observed in all cases of the induction of λ that we have studied.

DISCUSSION

These two strains which are mutant in two recombinational functions which seem to complement each other show differing behavior as regards the expression of the gene product of recA. Two anomalies exist between the yield curve for precA as observed by Kazanis and the behavior of the cells. These are for the absence of IIPRD in the recB strain, rather simply explained by invoking the absence of functional exonuclease V, and the absence of IRR in the recB. This is not so easily understood. Apart from these two anomalies the phenomena of septum inhibition and the induction of λ behave normally. This last does not fit the yield curve for precA, but nor does it in any other strain and it is most probable that the induction of λ has the derepression of recA as a prerequisite, but that an additional UV insult is needed. The same is true for these two strains. Thus one can say broadly that while there may be different "pathways" for induction in the two strains and that these show in the relations observed for the development of the recA gene product, the presence of precA is able to achieve much the same changes in the induced cells. The exceptional process, which invites careful consideration, is the induction of radioresistance. This is absent in induced recB cells, cells which will yield λ and show septum inhibition. It is also present in weak degree in recF cells which are induced and show all the other phenomena quite definitely. The best suggestion we can make is that the function of the inducible inhibitor of postradiation DNA degradation is to give a time "window" within which exonuclease V can perform needed DNA degradation and then close down the action by inhibition. Cells without X-ray damage do not need this turning off of the enzyme, for they are in no danger of losing too much DNA and the efficient recombination confers stability on the cells.

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If exonuclease V is missing and its place is taken by a hypothetical endonuclease which is not particularly responsive to the binding between precA and the DNA then no induced radioresistance would be seen. What is very interesting is the finding that even if exonuclease V is fully active and the recF putative endonuclease is not, the degree of induced radioresistance is minor. This is a problem we have not yet resolved.

One point of interest is that the existence of septum inhibition, thought to be needed for the cell to have time to get repaired genomes together and functional does not seem to be the basis for induced radioresistance for both these mutant strains show normal septum inhibition and yet are both abnormal in radioresistance.

ACKNOWLEDGEMENT

This work was supported by the U.S.Department of Energy, Contract No. EY-76-S-05-3631.

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APPENDIX 7

DOSE RESPONSE RELATIONS FOR UV INDUCTION OF SEPTUM INHIBITION IN MUTANT STRAINS OF *E. COLI* K12

Running Title: UV induction of septum inhibition in *E. coli*

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Abstract--Bacterial cells incubated for 90 minutes after exposure to graded doses of UV were heat-killed and photographed in a Petroff-Hausser chamber. Cells of length beyond the normal range for unirradiated cells were scored as septum-inhibited. Strain AB1157 (uvr$^+$lon$^+$) shows a dose-response induction of septum inhibition coordinate with other manifestations of induced repair. Strain AB1886 (uvr$^-$lon$^+$) shows the induction at 5 to 6-fold lower UV dose. Strain AB1889 (uvr$^+$ lon$^-$) is intermediate between these two, while the double mutant, AB1896 (uvr$^-$lon$^-$), is somewhat more sensitively induced than for the uvr$^-$ alone. The uvr$^-$ gene confers more sensitivity than the lon$^-$, which develops long filamentous forms at longer incubation times, but the effect of lon$^-$ is additive with uvr$^-$. The uvr$^-$ defect stops cell growth as well as septum formation beyond a critical UV dose. The lon$^-$ defect shows a higher spontaneous rate of septum inhibition than uvr$^-$ or wild type.

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INTRODUCTION

Induced repair, or SOS, is associated with a variety of induced effects: the induction of lysogenic phage (Lwoff, 1950), W-reactivation (Weigle, 1953; Defais et al., 1971), error-prone mutagenesis (Witkin, 1974), inhibition of post-irradiation DNA degradation (Pollard and Randall, 1973), radioresistance (Pollard and Achey, 1975; Smith and Martignoni, 1976), respiration halt (Swensen and Schenley, 1970), and septum inhibition (Satta and Pardee, 1978). If these inductions are coordinate, they should relate to a single event, probably the derepression of the recA gene product.

When exposed to a small dose of UV followed by incubation, E. coli strain B develops into long aseptate multinucleate cell forms, or filaments (Witkin, 1967). Witkin (1947) described a radiation-resistant mutant of E. coli strain B, strain B/r, which does not form filaments but does exhibit some inducible division delay. The resistant strain was later designated lon^+. The more sensitive filament-forming strain was designated lon^-, a recognition of the genetic basis of the defect in septum formation (Howard-Flanders et al., 1964: Adler and Hardigree, 1964). While both lon^+ and lon^- strains exhibit inducible division delay or septum inhibition (Satta and Pardee, 1978), the term "filament" is reserved for lon^- strains. The latter are induced to much greater lengths than lon^+ strains and also differ in response to the method of induction (Kantor and Deering, 1968) and in sensitivity to colony survival (Van de Putte et al., 1963). Kantor and Deering (1967) suggested that UV induces filamentation by producing pyrimidine dimers. More recently septum inhibition has been associated with the recA gene (Green et al., 1969; Inouye, 1971), and with protein X (Satta and Pardee, 1978).

This study compares UV induction of septum inhibition in five strains of E. coli K12 shown in Table 1. These represent different combinations of three genetic defects: (i) uvr, defective in excision repair, (ii) lon, defective in septum formation, and (iii) recA, defective in recombination. The four $recA^+$ strains represent the four possible combinations of the uvr and lon mutations.

Cultures were grown in Roberts' C-minimal salts (2gNH_4Cl, 6gNa_2HPO_4, 3gKH_2PO_4, 3gNaCl per liter) supplemented with 2g/l Casamino acids and 5g/l glucose (autoclaved separately). The cells were incubated at 37°C and iced at about 2 X 10^8/ml. They were irradiated in 8-ml quantities of cold growth medium in a 46-mm diameter silica dish. Irradiations were with 265-nm monochromatic light from a double water-prism monochromator. Transmitted intensity measurements during the irradiation provided for average UV dose calculation by a method previously described (Pollard et al., 1977). Three doses were given additively to the contents of each dish, with stirring between dose increments and with removal of 2.5-ml samples. One-half ml of each sample was transferred to a dilution blank of iced growth medium for a survival analysis of the immediate effects of the UV light. The remaining 2-ml portion of each dose sample was incubated in growth medium at 37°C for 90 min. These operations were conducted under yellow light to minimize photoreactivation.

After the 90-min. incubation the cells were again iced and a second, post-incubation survival plating sample (0.5 ml) was removed from each dose sample. A drop of each sample was placed directly on the grid region of a Petroff-Hausser Bacterial Counter (C.A. Hausser and Son, Phila., USA). Each sample was photographed under the high-dry objective of a compound microscope using 35-mm Panatomic-X film in a Leica camera attachment. At least five different photographs (containing from 30 to 300 cells each) were made for each sample. Enlarged prints were analyzed by measuring 80 to 350 individual cell lengths from each dose sample. Results were tabulated by cell length (in microns) and by UV dose. Survival samples were plated in 0.5-ml quantities on nutrient agar in dilutions from 10^{-2} to 10^{-7}. Duplicate experiments confirmed the results for all four uvr and lon strains: both sets of data are presented here. Results for recA represent a single trial.

RESULTS

Determining the proportion of septum-inhibited cells

The distinction between the uninduced and induced cell classes is based on

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an analysis of the distribution of cell lengths in both the unirradiated and irradiated samples in all five strains. In the recA strains, 90% of all cells in the unirradiated samples fall into the 1-, 2-, 3-, or 4-micron length classes. In each case, the 4-micron class accounts for a substantial fraction of all cells in the unirradiated samples. However, the 5-micron class accounts for only a small fraction (5%) of all cells in the unirradiated samples. Analysis of the changes in the proportion of cell lengths in a particular size class in relation to UV dose also indicates that 5 microns is the definitive length for septum inhibition in the $recA^+$ strains. While the 2-, 3-, and 4-micron classes account for decreasing proportions of the total sample population with increasing dose, the 5-micron and greater length classes account for increasing proportions with increasing dose: these are the induced cell lengths. By similar analysis in strain AB2463 ($recA^-$) the 6-micron length was found definitive for septum inhibition.

Duration of the incubation period

As a result of preliminary experiments on the time-rate changes in the proportion of septum-inhibited cells and in the differential reproductive capacities of inhibited and uninhibited cells the incubation period was set at 90 min. For strain AB1157 (lon^+) and AB1899 (lon^-) unirradiated samples vs strongly-induced samples (about $6J/m^2$) compared as follows: (i) the proportion of septum-inhibited cells peaks at about 60 min. and thereafter decreases, (ii) decrease in colony survival is a result of the greater reproductive capacity of the uninhibited cells relative to the inhibited cells in the irradiated samples, and (iii) longer incubation periods allow for inhibited cells to be more easily distinguished from uninhibited cells. Thus, while 60 min. appears to correspond to the peak fraction of septum-inhibited cells, this shorter time period makes it more difficult to distinguish between the two classes of cells, since most septum-inhibited cells are barely out of the normal cell length range. Incubation for 90 min. allows for one additional doubling time while the proportion of cells which are septum-inhibited is only 3-5% lower than at 60 min.

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Dose-response relations

A plot of the fraction of septum-inhibited cells against UV dose for the four uvr and lon combinations is shown in Fig. 1. The unirradiated samples (0-dose) contain a small proportion of septum-inhibited cells. Fig. 2 illustrates induction of septum inhibition in $recA^-$ shown with AB1157 (uvr^+lon^+) and AB1886 (double mutant) for comparison. The $recA^-$ strain shows an appreciable amount of septum inhibition inducible by UV, although considerably less than for the $recA^+$ strains.

In septum inhibition all-or-none?

If induction of septum inhibition is single-hit, an all-or-none effect, then from the Poisson distribution the fraction of cells which are induced should follow a relationship of the form $1 - e^{-DS}$, where D is the UV dose and S is a sensitivity parameter characteristic of the strain. A plot of the decrease in the proportion of normal length cells should be described by e^{-DS}. Fig. 3 reasonably confirms this relationship for the two uvr^+recA^+ strains. AB1157 and AB1899.

Survival and septum inhibition

The results of post-incubation colony survival as a function of dose is shown in Fig. 4. As expected, the strains differ markedly in their sensitivities to UV, primarily in relation to the uvr gene. In Fig. 4, the survival curves are compared with curves based on the changes in the proportion of normal-length (presumably uninduced) cells with dose. The plot of the proportion of normal length cells in the uvr^- strains shows that at higher UV dose these excision repair deficient strains stop growing and dividing.

Spontaneous induction

Even the unirradiated samples contain some small fraction of cells which are septum-inhibited, the fraction of cells which are induced without UV. The proportion of spontaneously-induced cells varies between strains as shown in Table 2.

DISCUSSION

Of the four $recA^+$ strains, AB1157 (uvr^+lon^+) is the least sensitive (requires

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the highest doses) for UV induction of septum inhibition. The induction of AB1157 closely resembles UV dose-response relations for W-reactivation, the induction of protein X, and several other induced effects (Kazanis et al., 1979). The introduction of the lon mutation, in AB1899 (uvr^+lon^-), markedly increases the sensitivity to UV. Even though both of these strains are capable of excision repair, the lon defect confers considerable additional sensitivity to UV. In AB1886 (uvr^-lon^+), lacking excision repair, the uvr defect confers even greater sensitivity than the lon defect, probably related to the numbers of dimers excised before reaching the replication fork. The double mutant, AB1896 (uvr^-lon^-), is the most sensitive of the four strains. Again, the lon mutation confers additional sensitivity to UV even in excision-repair deficient strains. Daughter strand gaps are not the only determinant of the induction of septum inhibition.

It is surprising that the $recA^-$ strain, generally deficient in induced repair, shows some degree of inducible septum inhibition. At very low doses, the induction of $recA^-$ closely resembles the $recA^+$ strains, but a higher doses the fraction of elongated $recA^-$ cells is never greater than 0.5.

Deering (1958) suggested that induction of filamentation was an all-or-none phenomenon. The data for AB1157 (uvr^+lon^+) and AB1899 (uvr^+lon^-) (Fig. 3) support that suggestion. The determination of this relationship in uvr^- strains is complicated by the survival sensitivity of these strains at higher doses which leads to a maximum and decline in the proportion of elongating cells with increasing dose.

As expected, the post-incubation survival analysis for the four $recA^+$ strains shows that the uvr gene primarily determines the colony-forming ability of these strains; uvr^- strains are considerably more sensitive. This uvr effect is only slightly mofified by the lon gene. Clearly, there is a relationship between colony-forming sensitivity and the fraction of uninduced or non-septum-inhibited cells (Fig. 4). The fraction of uninduced cells closely parallels the survival ratios in a given strain (i.e., most filaments do not form colonies). However, the fraction of uninduced cells shows greater dependence on the lon gene than does

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colony formation. The change in slope in the fraction of cells which are not septum-inhibited in the uvr$^-$ strains is probably a reflection of the extreme sensitivity of these strains to killing at higher doses, a failure of excision repair inhibiting cell growth at higher doses.

The fraction of spontaneously-induced cells (septum-inhibited in unirradiated samples) is notable for all five strains. This fraction depends considerably upon the lon gene; lon$^-$ strains have a higher level of spontaneous induction than lon$^+$ strains (between 7 and 8%). Since septum inhibition is believed linked to the induction of protein X, the degree of spontaneous induction may reflect the level of protein X resulting from the spontaneous derepression of the recA gene. The relatively high levels of spontaneous induction suggest that the binding of a repressor to the recA operator is much weaker than would be inferred from the low probability of spontaneous induction of phage λ. The sigmoidal form of UV dose response relations for λ-induction suggests that the induction of λ is a multiple-hit phenomenon (Fluke and Pollard, 1979) which these results for septum inhibition tend to confirm.

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Acknowledgements--Dawn Jenkins assisted in the analysis of the length distribution data. This work was supported by the U.S. Department of Energy Grant EY-76-S-05-3631.

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Table 1. Comparison of radiation-related mutations in five strains of *E. coli* K12. The recA strains represent the four possible combinations of the *uvr* and *lon* mutations.

STRAIN	uvr	lon	excision repair	filaments
AB1157	+	+	+	-
AB1899	+	-	+	+
AB1886	-	+	-	-
AB1896	-	-	-	+
AB2463	recA$^-$		recombination deficient	

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Table 2. Proportion of spontaneously-induced cells. The fraction of septum-inhibited cells in the unirradiated samples are shown for the five strains. Data represent the average of duplicate experiments for the four uvr and lon strains. Results for recA represent a single trial.

STRAIN	FRACTION OF CELLS SPONTANEOUSLY INDUCED
uvr^+ lon^+	.030
uvr^- lon^+	.033
uvr^+ lon^-	.080
uvr^- lon^-	.070
$recA^-$	.035

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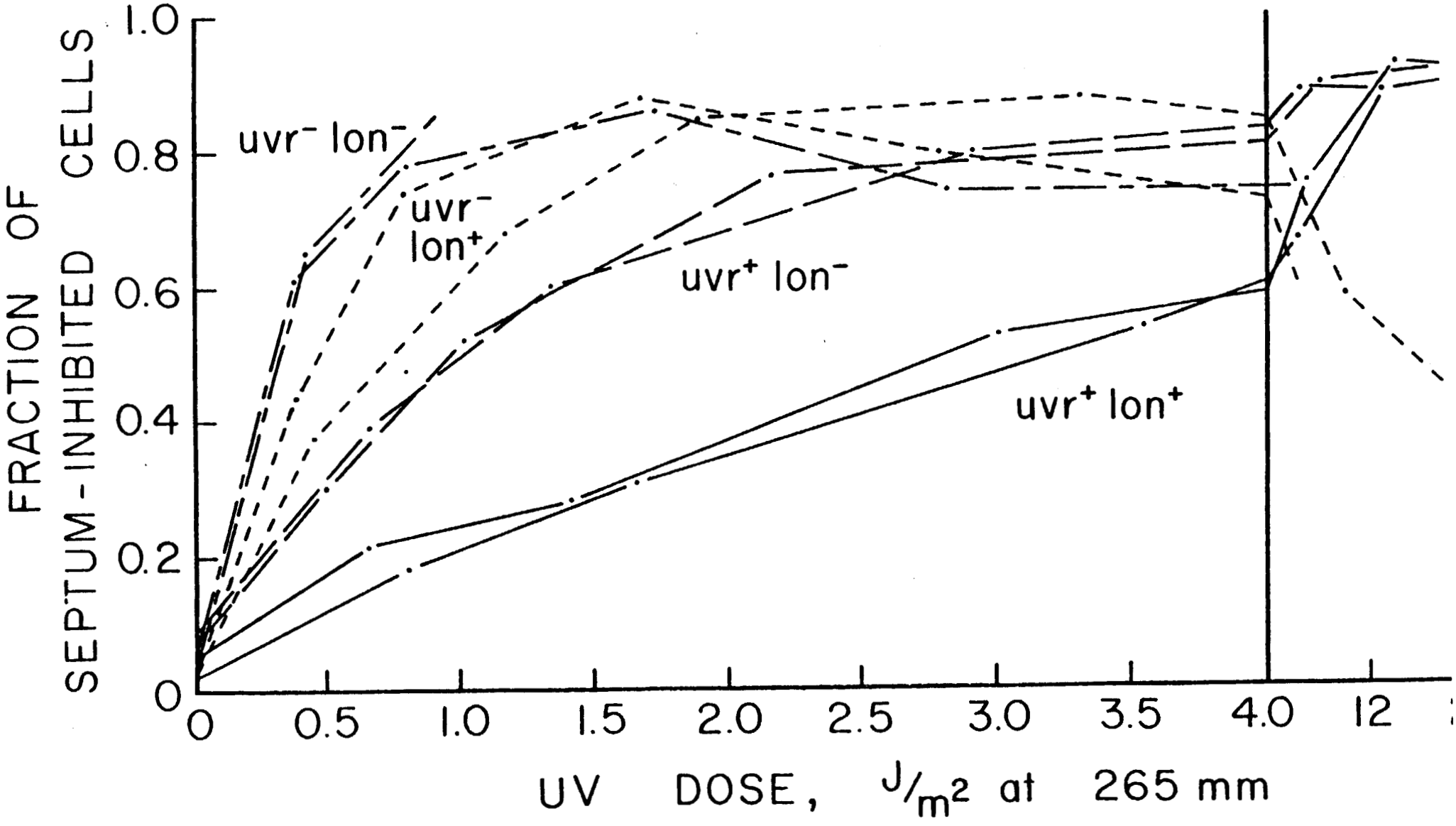

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Figure 1

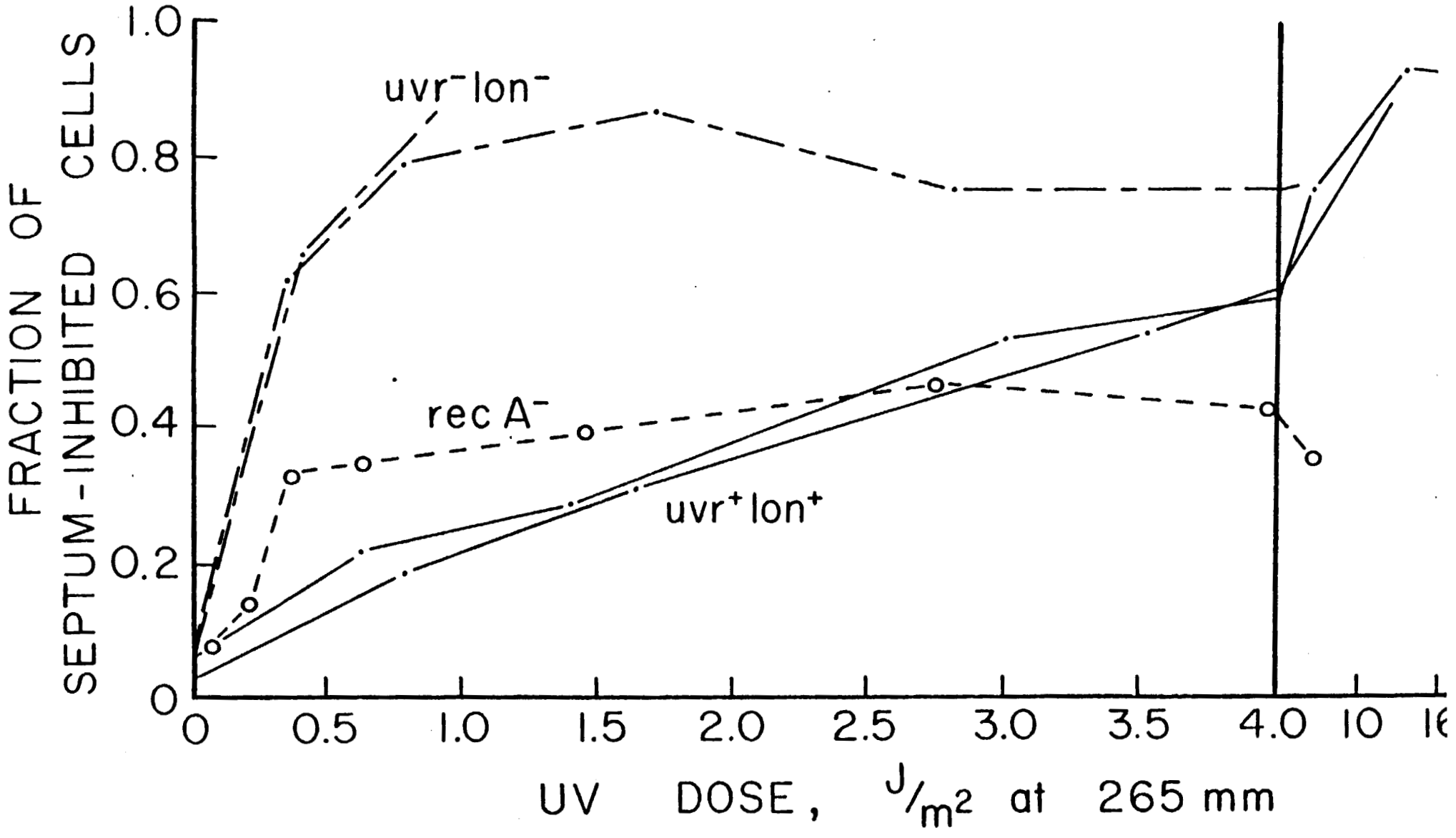

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Figure 2

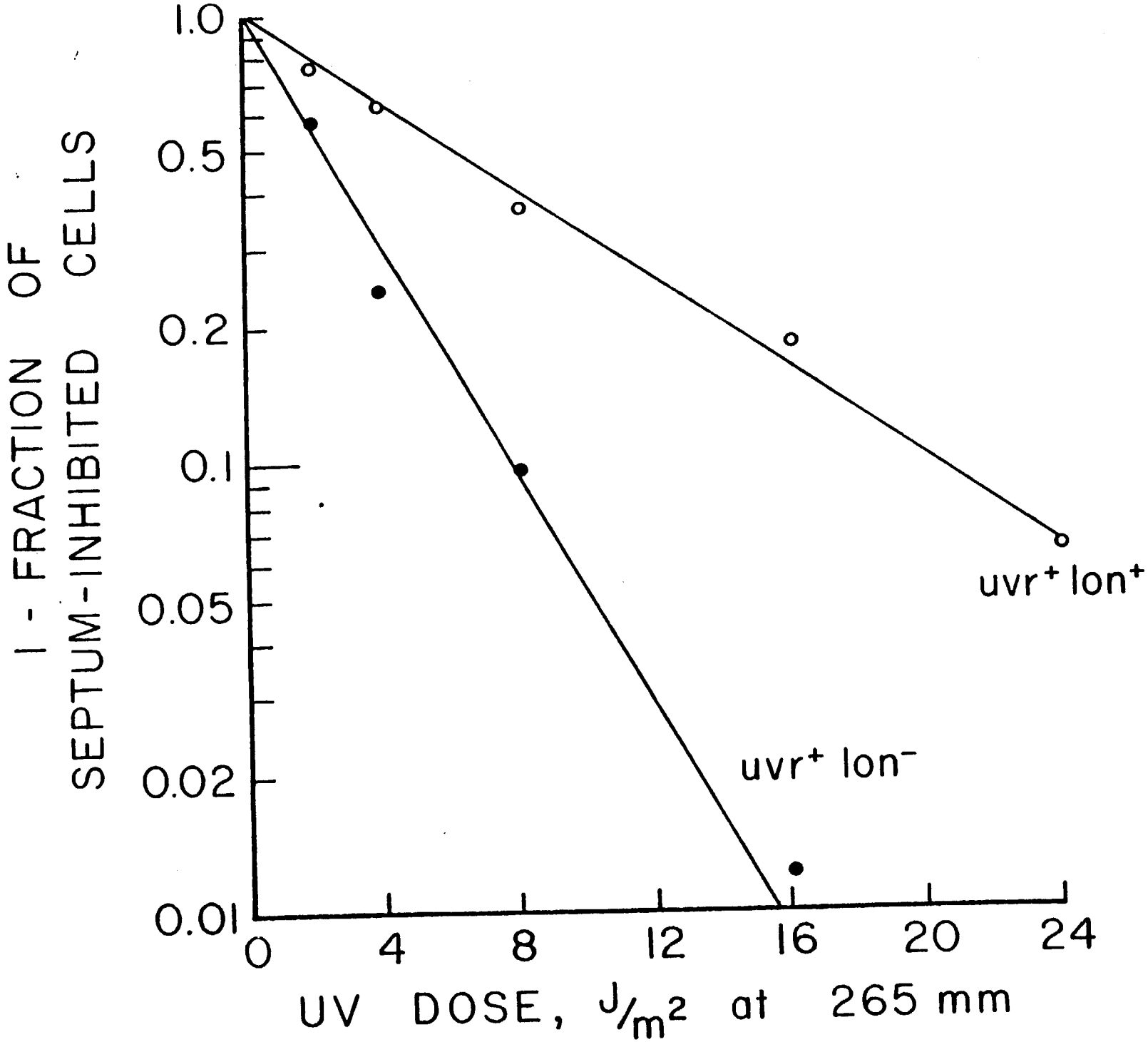

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Figure 3

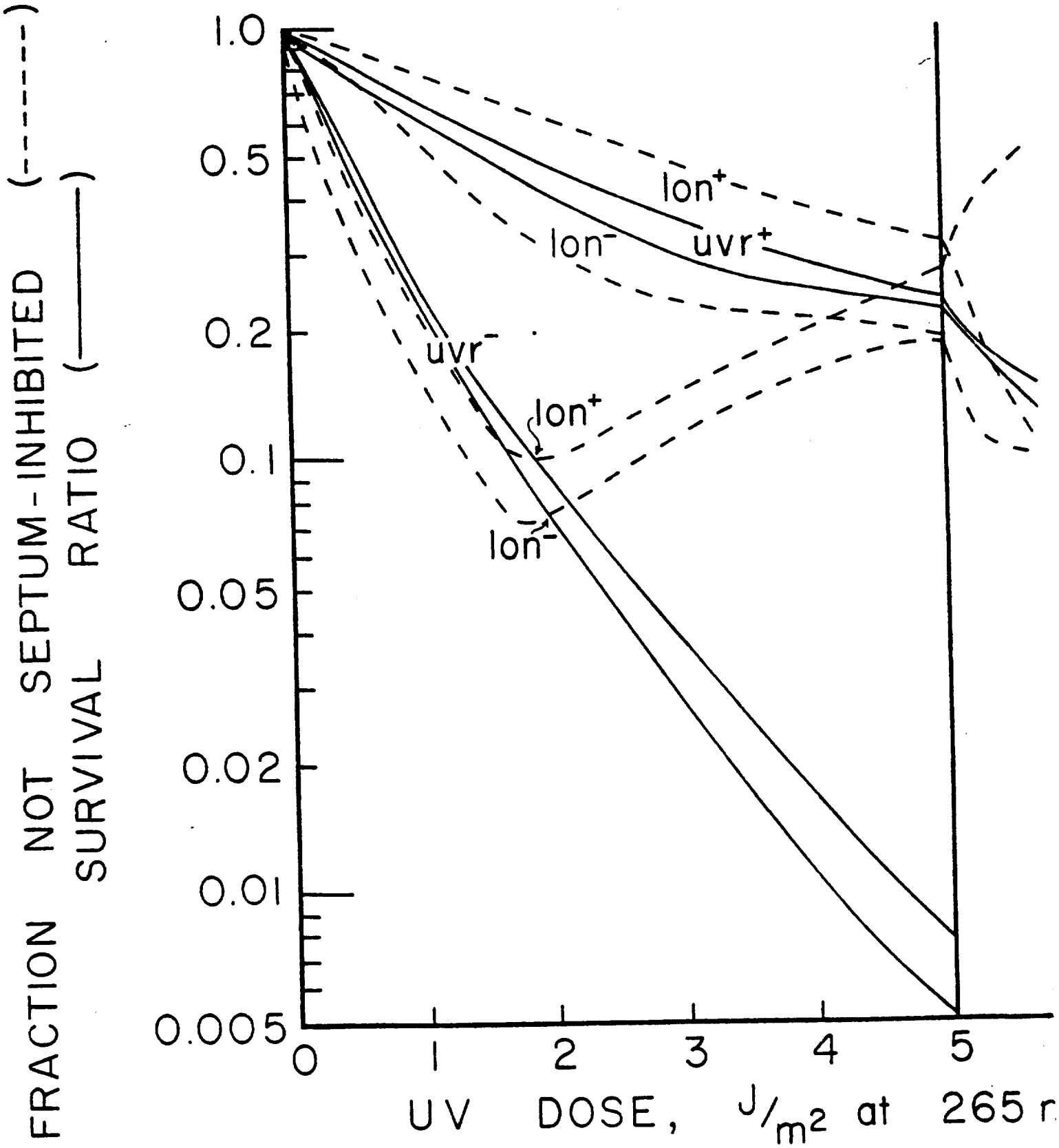

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Figure 4

Figure 1. Dose response relations for uvr and lon strains. Two trials are shown with data points for each strain. The scale changes at 4.0 J/m^2 in order to expand the initial portions of the curves.

Figure 2. Dose response relations for the $recA^+$ strain. Data for uvr^+lon^+ and uvr^-lon^- are reproduced from Fig. 1 for comparison. The scale changes at 4.0 J/m^2 as in Fig. 1.

Figure 3. All-or-none character of septum inhibition. The proportion of normal length cells in AB1157 (lon^+) and AB1899 (lon^-) follows the Poisson distribution described by e^{-DS}, where D is the UV dose and S is a sensitivity parameter. Data points represent the average of duplicate experiments.

Figure 4. Dose-response relations for colony survival and the proportion of normal length cells. The curves are drawn from the average of two trials, data points omitted. The scale change at 5 J/m^2 expands the initial portion of the curves.

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APPENDIX 8.

THE EFFECT OF INDUCTION OF THE INDUCED REPAIR SYSTEM WITH NALIDIXIC ACID ON THE YIELD OF LEUCINE REVERTANTS PRODUCED BY UV LIGHT.

Anne Coleman, D.J.Fluke and E.C.Pollard

INTRODUCTION

A great deal of evidence has accumulated to indicate that for some UV mutagenesis it is necessary to induce the induced repair system and also to produce the pre-mutagenic lesion. This has been discussed more than once in the previous material. A very potent derepresser of recA is nalidixic acid. It is also a very feeble mutagenic agent. It should therefore be possible to induce the induced repair system with nalidixic acid, permit the precA to develop and then to find an increased yield of mutations due to UV and also an increase in their number proportionally to the dose rather than requiring a square law to describe their behavior. This has been approached by Doudney (1) who showed the change from quadratic to linear for reversion from tryptophan auxotrophy in strain WP2 thy trp. He found this to occur in the uvr^+ strain but not in the uvr^- . Our work has mostly been done with strain WU3610 89, which is leu^- tyr^- and uvr^-. In view of our considerable experience in studying leucinerevertants with this strain it seemed sensible to see if the transition from quadratic to linear could be observed after nalidixic acid treatment.

The technique has been described before. It was followed with very little change. Routine provision for estimating the number of mutants previously in the culture was made by plating the unirradiated cells at three dilutions. Any observed effect of the dilution is ascribed to mutants brought in with the culture and not to spontaneously arising mutants. Nalidixic acid was given at 0.4microgram per ml final concentration, allowed 30 minutes at 37°C to take effect and then removed by centrifugation. The irradiation and plating procedures were as previously described. One set of data were taken

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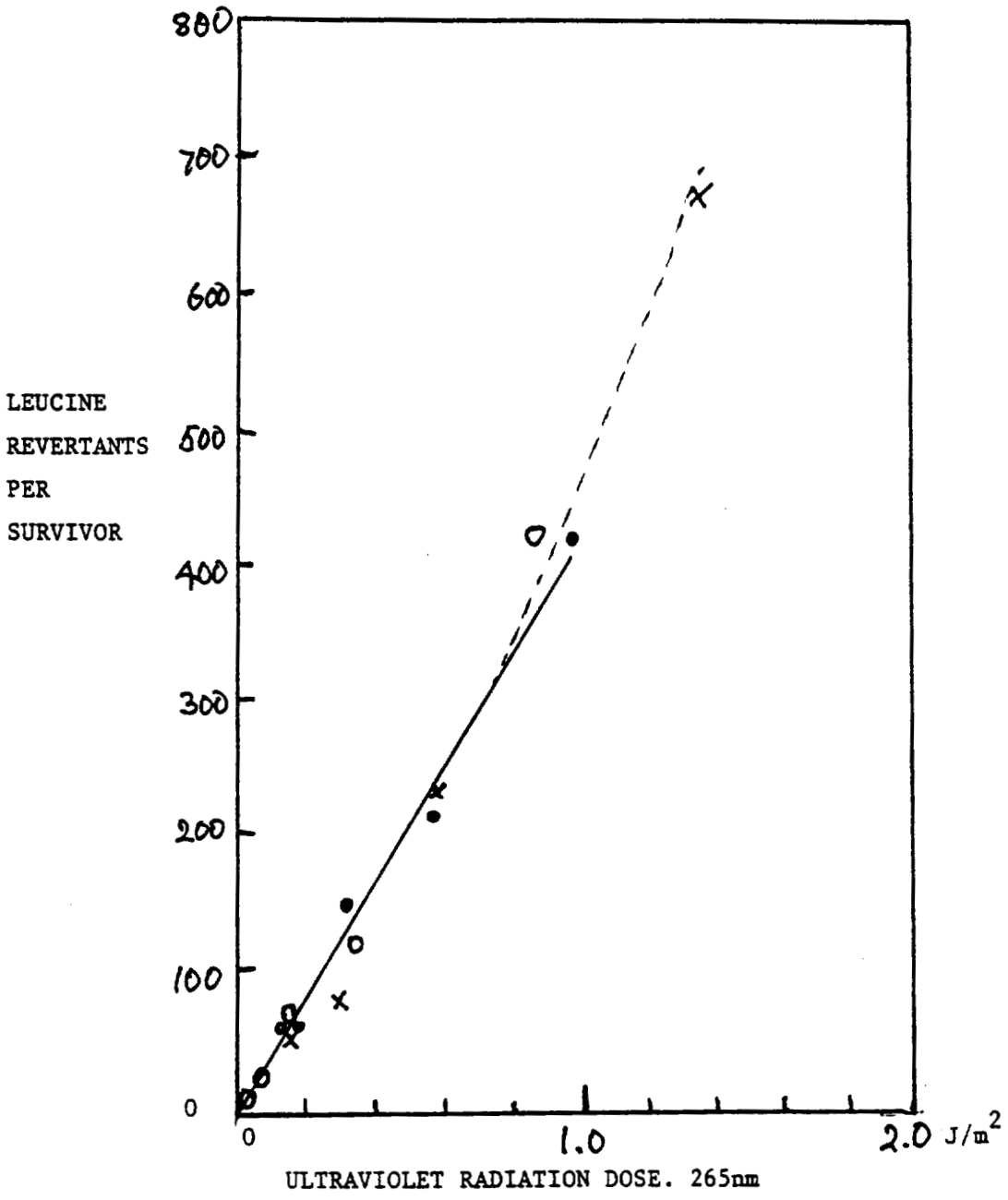

Figure 9.1 Leucine revertants produced by exposure to UV light in cells of strain WU3610 89 which have been previously treated with nalidixic acid to cause derepression of recA. The induced cells respons to UV light by producing revertants more nearly proportional to the dose than obeying the quadratic dose relationship. Thus induced repair is involved in UV mutagenesis.

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without previous treatment with nalidixic acid. These form part of the analysis in Appendix 2. Of considerable interest is the fact that when the revertants per survivor are divided by the observed yield of precA measured by Kazanis with the same absolute dosimetry, the resultant is a very good linear proportionality with the dose. Thus, in this instance, if the yield of revertants per survivor, R/S is supposed to be a compositefunction of a premutagenic lesion, the numbers of which are proportional to the dose, and an inducing function: i.e.

$$R/S = k_1 D\ f(D)$$

Then f(D) is precisely the same as the induction yield expression for the recA gene product. This is true in this strain WU3610 89. It is not necessarily always true. However, it is excellent evidence that induced repair, and particularly the recA gene product does have a role in mutagenesis.

When previous treatment with nalidixic acid has been given the relationship of Figure 1 is seen.The revertans per survivor aresomewhat higher at lower doses than for no nalidixic acid and the relation to dose is much more nearly linear. Most of the experimental work was done at quite low doses. At quite high doses there is some anomaly, not further studied here.

These experiments support the idea that if the induced repair system is actively in the cells, as produced by nalidixic acid, then the only requirement for mutation is the premutational lesion, which is proportional to dose. It is likely that 100% induction was not achieved by nalidixic acid, for there is an upswing beyond the linear apparent around $1J/m^2$. These experiments were performed in a uvr^- strain, so Doudneys observation that no linearization with nalidixic acid is to be seen in uvr^- does not seem to be a general conclusion.

INDUCED REPAIR IN E.coli: INDUCTION BY ULTRAVIOLET LIGHT AT -79°C.

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INTRODUCTION

The phenomenon of induced repair, also called S.O.S. repair and error-prone repair, is found in cells of E.coli that are recA$^+$ and lexA$^+$. It reveals a pleiotropic response to the induction of the recA gene product (1). Three of its manifestations are: induced inhibition of post-radiation DNA degradation, induced radioresistance, and the induction of phage λin λ lysogens. All are readily induced by ultraviolet light under normal conditions, and are diminished when exposed to photoreactivating light. Ultraviolet light given to cells which are frozen at Dry Ice temperatures has been shown to produce a different range of effects. (2,3). The cells are more sensitive and UV light is more mutagenic. The question arises as to whether exposure to UV under these conditions is able to elicit induced repair, and if so, whether it is photoreactivable. Of further interest in this kind of irradiation is the suggestion that one major form of UV damage under the frozen conditions is in the form of DNA-protein cross-links. (4,5).

We find that the induced inhibition of postradiation DNA degradation is readily induced under these circumstances and is not markedly photoreactivable. Induced radioresistance is also readily induced and is not photoreactivable. The induction of phage-λ is complicated by the sensitivity of the λ-lysogen to freezing, but there is some indication that in those cells which survive freezing, UV on the frozen cells is an efficient inducer.

MATERIALS AND METHODS

The bacterial strains used were E.coli WU3610, WU3610-89, K12, and K12λ. The first two were obtained originally from Dr. Evelyn Witkin by Dr. Person.

Cells were grown in Roberts C-minimal medium (2gNH_4Cl, 6g Na_2HPO_4, 3g KH_2PO_4, 3g NaCl, 120mg $MgCl_2$, 60mg Na_2SO_4 per liter) supplemented by 0.2% casamino acids and with 5g/liter glucose. This is referred to as casaC medium. The cells were frozen in dishes on a Dry-Ice- methanol mixture, or alternatively on Millipore filters kept at Dry-Ice temperatures in a metal dish. Comparative irradiations in the wet state were

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performed in a silica dish with the transmitted ultraviolet light monitored for intensity averaging. (1). The Millipore-filter preparations were resuspended by putting the filter in a 100ml beaker and adding the casaC medium. The recovery of unfrozen cultures approaches 100%; and the recovery of the frozen cells was about 50% except for K12λ, where the recovery is about 10% or less.

For the induced inhibition of post-radiation DNA degradation the cells were labeled with 200μg deoxyadenosine per ml, 1μg cold thymine per ml and 0.5μCi/ml ^{3}H-thymidine. UV exposure was followed by 40 minutes of incubation at 37°C, addition of 50μg per ml rifampin and ten more minutes of incubation. They were then given between 20 and 30 kilorads of X-or gamma rays. The solubilization of the DNA in cold TCA was observed as a function of time of incubation after irradiation. Photoreactivation was performed with either a bank of BLB "black light" or fluorescent white lamps.

For induced radioresistance the same procedure was followed, except that graded doses of X- or gamma rays were given with plating after each dose.

For the induction of λ two methods were used. The first involved the observation of the development of endolysin with lysis of the cell wall. Cells were grown in lactose, to induce β-galactosidase. They were then irradiated as above. At various times they were smapled into reaction mistures of ONPG, the color substrate for β-galactosidase. (6). They were then held at 31°C for standard times, (short, if cells were undergoing lysis and longer otherwise), and the yellow color measured. If λ is induced there is a great increase in yellow color around 70 minutes. The technique has been described by Coetzee and Pollard (6). Additionally to this, after UV exposure the cells were assayed for plaque development at 0,45, 80, and 110 minutes. The first two plaque assays measure infective centers and the last two the free phage in a burst. For estimating the number of cells induced from the observation of free phage, a burst size of 65 was assumed.

RESULTS

Figure 1 shows the effect of UV pre-treatment of strain WU3610-89 in the wet state and in the deep frozen state, with and without photoreactivation. The observation is of the inhibition of post radiation DNA degradation; the time course of DNA degradation after 25krads γ-radiation is shown. There is inhibition of DNA degradation because of the pre-treatment with UV light. The inhibition is less after photoreactivating treatment for UV light given in the wet state, while there is no difference for UV given in the frozen state.

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Also, in the frozen state there is more efficient inhibition.

Figure 2 shows the effect of UV light in developing radioresistance in strain WU3610. UV given in the wet state gives good induced radioresistance which is substantially reduced by photoreactivating treatment. In contrast, UV light given in the frozen state produces an effect which is not photoreactivatable. In the uvr$^-$ strain WU3610-89 we observed the development of induced radioresistance after 0.8J/m^{2}UV both wet and in the frozen state. We did not test pnotoreactivability. (Data not shown).

For the induction of λ-phage in strain K12λ we found the recovery of cells frozen on Millipore filters, and, in fact, frozen on Dry Ice in any way, to be markedly less than for non-lysogens. Instead of 50% recovery there was less than 10% and this amount was quite variable, making the whole experimental procedure very unsatisfactory. The endolysin assay showed that the cells which had not been treated with UV at all developed 50% of maximum enzyme activity which was produced in the first 45 minutes of incubation or even less. Freezing K12λ evidently produces some damage to the cell wall and membrane which is deleterious to cells. Our best experiments under these very difficult conditions suggested that the surviving K12λ are induced by UV light in the frozen state and that induction is not photoreactivable.

DISCUSSION

That UV light given at low temperatures in the frozen state produces DNA-protein cross links has been suggested by Bridges, Ashford-Smith and Munson (4) and by Smith and O'Leary(5). In addition, Smith and Hamelin (7) have shown that damage due to UV in the frozen state causes relatively more inhibition of DNA synthesis and also that post-replication repair can occur on damage produced in the frozen state.

Our findings indicate that while UV in the frozen state is potent in damaging cells it is also an efficient inducer of two of the phenomena associated with induced repair.

ACKNOWLEDGEMENTS

We acknowledge support from the Energy Research and Development Administration grant E(11-1) 2362.

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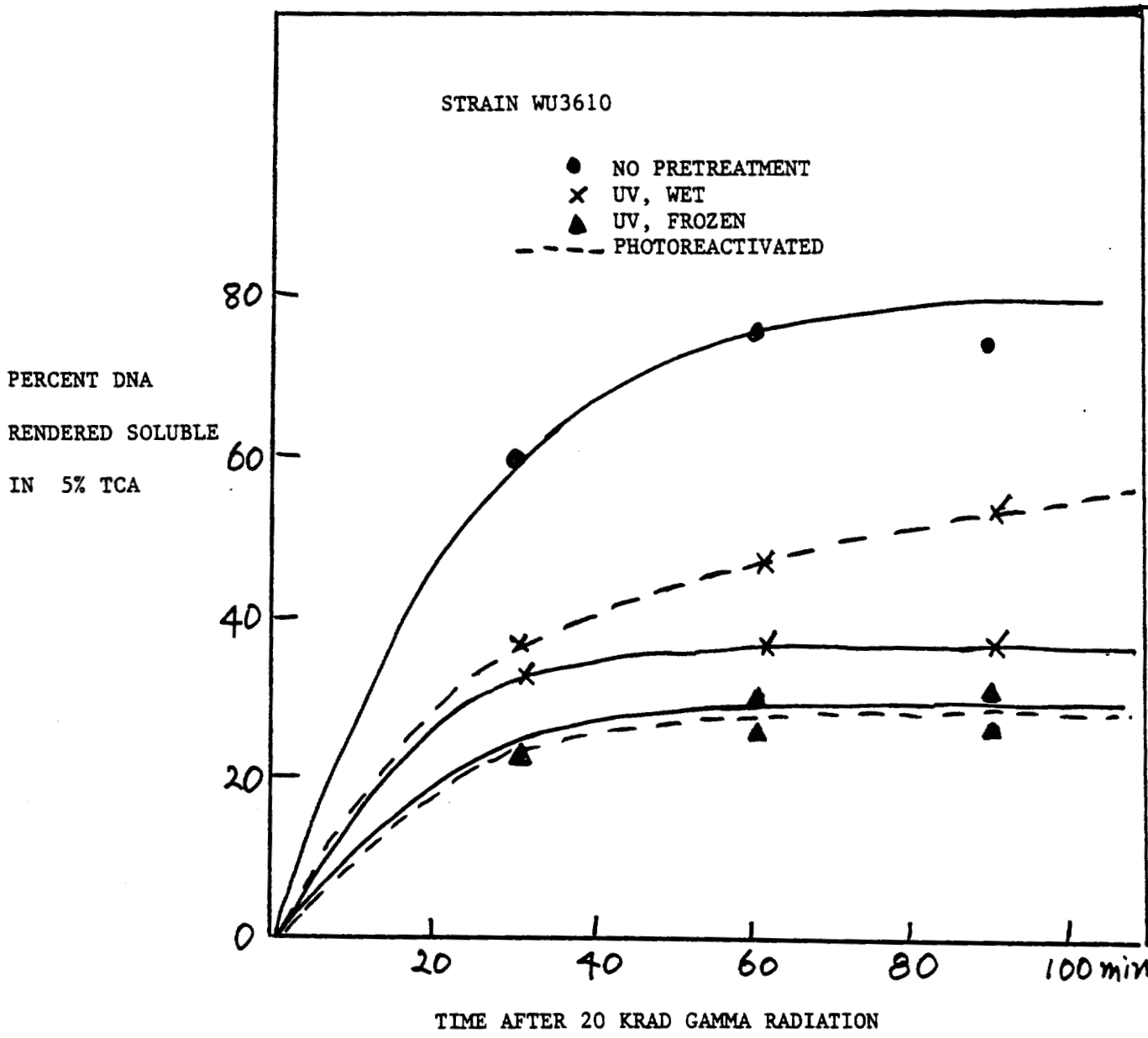

Figure 9.1 The effectiveness of UV given at -79°C in causing the induction of the inhibition of post irradiation DNA degradation. Untreated cells reach 80% degradation. Those which have been previously irradiated in the wet state show much less, but this inhibition is diminished after exposure to photoreactivating light. For cells previously irradiated frozen the inhibition is greater and there is no effect of photoreactivating light.

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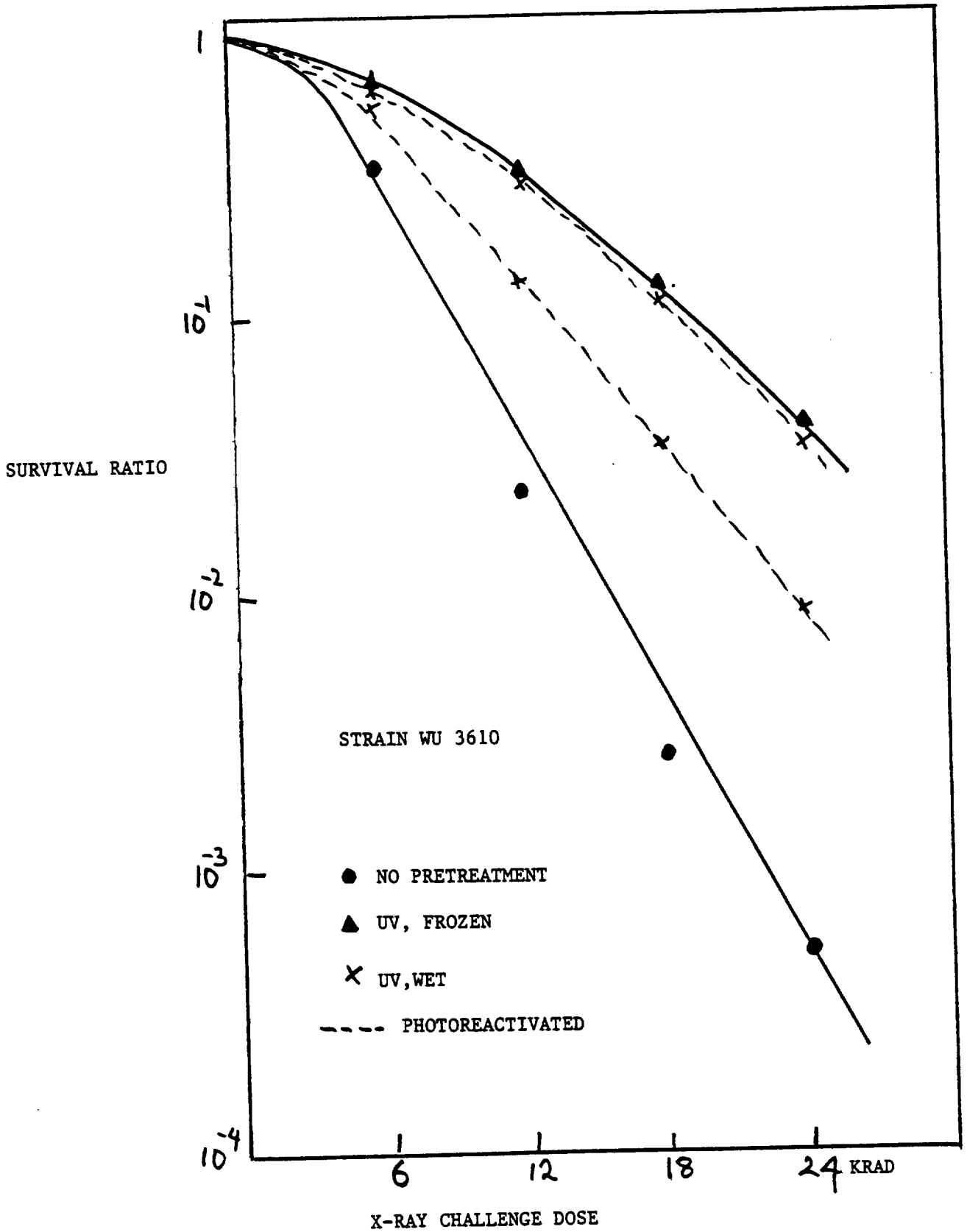

Figure 9.2 The induction of radioresistance by giving UV at -79°C. The pretreated cells are clearly more resistant whether the UV is given wet or frozen. However, the cells which have received UV in the frozen state are not changed by exposure to photoreactivating light, while those exposed in the wet state are.

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APPENDIX 10

Independent Study Progress Report

Richard L. Page

Resistance to the killing effects of X-rays has been induced in E. coli. My goal was to see if X-ray resistance could be induced in Chlamydomonas reinhardi. The first step was to give a simple series of graded X-ray doses in order to find the basic survival curve I would be working with. A dish containing 6.0 ml. of solution of 1 X 10^7 cells/ml. was exposed to a total of 30 krad., with samples removed after each multiple of 6 krad.

My first experiment looked for X-ray resistance induced by a pre-dose of X-rays. A procedure similar to that for the first survival curve was performed on a control sample of solution, while a second 6 ml. sample was given a "pre-tickle" of 6 krad and then allowed to incubate for 120 minutes. To both samples was added 0.2 ml. of cyclohexamide solution (3.0 X 10^{-4} g/ml.) ten minutes prior to the exposure to the graded doses.

The survival curves for the fractionated and non-fractionated dose samples did not differ significantly from each other or from the first X-ray survival curve. X-ray resistance was not induced by this technique.

At this point I decided to move on to investigating if X-ray resistance could be induced by ultra-violet radiation. My first step was to perform a series of graded U.V. doses (ranging from 1 to 1000 J/m^2) on a solution sample in order to yield a survival curve. The 5.0 ml. sample contained 1.0 X 10^7 cells/ml., and the doses were given using the monochrometer. In this and all following experiments employing ultra-violet radiation, only yellow lights were used, and the plated samples were kept in the dark for at least 12 hours following irradiation. In this way, any photoreactivation is avoided. The survival curve gave an idea of the dose to give as the mutation inducing dose for later experiments.

In my first attempt to induce X-ray resistance with U.V. radiation, a 5.0 ml. sample of Chlamydomonas reinhardi (1.0 X 10^7 cells/ml., as in all following trials)

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was exposed to a U.V. dose of 31.3 J/m^2 and allowed to incubate for 110 minutes. Cyclohexamide was again added to both the U.V. exposed sample and to the control sample ten minutes before both samples were exposed to the graded X-ray dosed.

The resulting survival curves from this experiment, seemed to indicate that resistance was induced, but to be sure, these results must be reproduced.

The second trial involved a larger ultra-violet pre-dose than originally desired (100 J/m^2), but otherwise the procedure was the same as in the first trial. The resulting survival curves were very different from the first trials, however. The control and the pre-dosed samples gave almost superimposable curves. The result warranted still another trial of the experiment.

The third trial involved three samples: a control and two samples exposed to U.V. pre-doses (one sample was exposed to 27.8 J/m^2 and the other was exposed to 57.0 J/m^2). The rest of the procedure remained the same as in the first two trials.

In this trial, the ultra-violet radiation seemed to sensitize the cells to the X-ray killing effects, rather than causing resistance. The larger U.V. dose seemed to sensitize the sample more than the smaller dose. In any case, X-ray resistance was definitely not induced.

In conclusion, induction of X-ray resistance does not seem to have been achieved in my experiments. Such resistance may well be inducible, but by some other means than those employed in the above studies.

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X-RAY SURVIVAL CURVE

X-ray Dose (krad.)	Number Surviving	Fraction Surviving
0	7.37×10^{6}	1.00
3	5.05×10^{6}	6.95×10^{-1}
6	3.66×10^{6}	4.97×10^{-1}
9	2.54×10^{6}	3.45×10^{-1}
12	7.95×10^{5}	1.08×10^{-1}
15	1.77×10^{5}	2.40×10^{-2}
18	1.04×10^{5}	1.41×10^{-2}
21	3.18×10^{4}	4.31×10^{-3}
24	4.02×10^{4}	5.45×10^{-3}
27	1.84×10^{4}	2.50×10^{-3}
30	7.50×10^{3}	1.02×10^{-3}

Experiment I

	Control		Pre-Dosed (6 krad.)	
X-ray Dose (krad.)	Number Surviving	Fraction Surviving	Number Surviving	Fraction Surviving
0	4.11×10^{6}	1.00	2.86×10^{6}	1.00
6	2.19×10^{6}	5.33×10^{-1}	8.32×10^{5}	2.90×10^{-1}
12	2.87×10^{5}	6.90×10^{-2}	2.35×10^{5}	8.24×10^{-2}
18	7.12×10^{4}	1.73×10^{-2}	7.34×10^{4}	2.57×10^{-2}
24	8.17×10^{3}	1.99×10^{-3}	1.99×10^{4}	6.96×10^{-3}
30	1.32×10^{3}	3.22×10^{-4}	3.42×10^{3}	1.20×10^{-3}

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U.V. SURVIVAL CURVE

Dose (J/m^2)	Number Surviving	Fraction Surviving
0	4.98×10^6	1.00
1.07	1.54×10^6	3.10×10^{-1}
2.24	3.68×10^6	7.40×10^{-1}
5.52	2.98×10^6	5.98×10^{-1}
11.0	4.10×10^6	8.25×10^{-1}
22.0	4.08×10^6	8.19×10^{-1}
56.0	3.30×10^6	6.63×10^{-1}
116	1.11×10^6	2.23×10^{-1}
216	2.83×10^5	5.69×10^{-2}
552	9.93×10^2	2.00×10^{-4}

Experiment II, Trial 1

	Control		Pre-Dosed ($31.3\ J/m^2$)	
X-ray Dose (krad.)	Number Surviving	Fraction Surviving	Number Surviving	Fraction Surviving
0	7.68×10^6	1.00	3.80×10^6	1.00
6	8.05×10^5	1.05×10^{-1}	8.88×10^5	2.34×10^{-1}
12	1.32×10^5	1.72×10^{-2}	9.05×10^5	2.38×10^{-1}
18	3.14×10^4	4.44×10^{-3}	1.48×10^5	3.89×10^{-2}
24	1.44×10^4	1.88×10^{-3}	9.22×10^4	2.43×10^{-2}
30	2.08×10^4	2.70×10^{-3}	2.20×10^4	5.78×10^{-3}

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EXPERIMENT II, TRIAL 2

X-ray Dose (krad.)	Control: Number Surviving	Control: Fraction Surviving	Pre-Dosed (100 J/m^2): Number Surviving	Pre-Dosed (100 J/m^2): Fraction Surviving
0	1.54×10^7	1.00	4.67×10^6	1.00
6	2.88×10^6	1.88×10^{-1}	1.00×10^6	2.15×10^{-1}
12	6.90×10^4	4.50×10^{-3}	4.38×10^4	9.37×10^{-3}
18	3.40×10^3	2.21×10^{-4}	1.30×10^3	2.40×10^{-4}
24	3.50×10^2	2.28×10^{-5}	**	
30	**		**	

("**" signifies that an insufficient number of cells for counting survived.)

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EXPERIMENT II, TRIAL 3

X-ray Dose (krad.)	Control Number Surviving	Control Fraction Surviving
0	9.36×10^{6}	1.00
6	1.95×10^{6}	2.08×10^{-1}
12	4.39×10^{5}	4.69×10^{-2}
18	1.33×10^{5}	1.42×10^{-2}
24	7.73×10^{3}	8.25×10^{-4}
30	3.45×10^{3}	3.68×10^{-4}

X-ray Dose (krad.)	27.8 J/m^2 Pre-Dosed Number Surviving	27.8 J/m^2 Pre-Dosed Fraction Surviving	57.0 J/m^2 Pre-Dosed Number Surviving	57.0 J/m^2 Pre-Dosed Fraction Surviving
0	7.12×10^{6}	1.00	6.25×10^{6}	1.00
6	2.92×10^{6}	4.10×10^{-1}	2.48×10^{6}	3.81×10^{-1}
12	2.37×10^{5}	3.33×10^{-2}	1.90×10^{5}	2.92×10^{-2}
18	6.48×10^{3}	9.10×10^{-4}	5.70×10^{3}	8.74×10^{-4}
24	8.50×10^{2}	1.19×10^{-4}	**	
30	3.5×10^{2}	4.90×10^{-5}	**	

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PUBLICATIONS

Reprints are enclosed of two publications which have appeared during the current year.

1. Dose response relations for UV induced induced repair phenomena.

In "DNA Repair Mechanisms" Ed. Hanawalt, Friedman and Fox. Academic Press 1978.

2. Relative rates of repair of single-strand breaks and postirradiation DNA degradation in normal and induced cells of Escherichia coli.

E.C.Pollard and James K.Fugate, Jr. Biophysical Journal 24 429 -437.

Other writing

As of the end of July 1979 no articles are in the editor's hands. The first of the series on "Contemporary Biophysics" edited generally by one of us (E.C.P.) has appeared. It is "The Aqueous Cytoplasm" edited by A.D.Keith. The first chapter in this volume "Relation of Synthetic processes in the Cell to cytoplasmic viscosity" is by Pollard. It is interesting for the use made of the radiation data of Harm and Rupert to calculate cytoplasmic viscosity. No reprints are available. The chapter in the CRC book on Radiation Sterilization is at the printers.

Immediately ready to submit, awaiting only figure completion are appendices 7 and 9. These will be submitted within days.

Appendix 2, the first of two articles on coordinate induction is ready for final review in our group. It will be submitted during August.

The paper on the separation of the premutagenic lesion and the inducing lesion cross sections, mentioned in Appendix 3 is being worked up. No further experimental work is contemplated.

Appendix 4 is ready for final rewriting and in-group review.

Appendices 5 and 6 need more work to make them more general.

A major, and we believe creditable reason for the holding back on publication has been the availability of excellent yield data on the recA gene product, due to Dr. Kazanis. Thus the content of Appendix 2 has been greatly strengthened by the addition of the information he has generated.

The book, second in Dekker's series on "Induced Repair" is 3/4 complete in first draft.

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