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Factors Affecting the Survival of *Escherichia coli* After Ultraviolet Irradiation

By

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TABLE OF CONTENTS

Page

INTRODUCTION 1

MATERIAL AND METHODS 6

RESULTS AND DISCUSSION

Effect of culture medium 8

Effect of pH of the plating medium 16

Effect of visible light 22

SUMMARY 30

ACKNOWLEDGEMENTS 32

APPENDIX I 33

APPENDIX II 45

REFERENCES 49

INTRODUCTION

The effects of radiation on living cells are often greatly modified by relatively slight changes in the physical and chemical conditions of an experiment. An increasing number of studies of factors of this kind have been made in recent years and new properties have been revealed for many classical radiation effects such as chromosome breakage, production of mutations, temporary inhibition of division and killing. It can be expected that further exploration of the influence of these factors will greatly improve the possibility of an exact understanding of the mechanisms of radiobiological action.

Considerable activity of this sort has been directed to studies of the action of radiation on bacteria and much of it has centered on the lethal effect of ultraviolet light on *Escherichia coli*. This introduction and the experimental work to follow will be largely confined to the bactericidal effect of ultraviolet radiation on strain B of this organism. A brief survey of the important factors known to influence the survival of *Escherichia coli* B after irradiation follows.

Selection of strain. In 1946 and following in 1947 Witkin reported the discovery of a mutation to radiation resistance in *Escherichia coli* B. In semi-logarithmic plot the survival curves of the mutant, B/r, and the parent strain, B, were respectively upwardly convex and upwardly concave with B/r generally requiring several times the dose required by B for a given degree of killing. B and B/r also differed in their sensitivity to X-rays. Exponential

survival curves were obtained with each but B/r was about one-half as sensitive as B.

Two reports have been made of gradual increases in resistance in E. coli resulting from repeated sub-lethal doses of ultraviolet light (Rentschler, Nagy and Mouromseff 1941 and Luckiesh and Knowles 1948). In both cases it was at least partially implied that these were not mutational changes. If such changes can occur, knowledge of the history of a strain with respect to radiation would be neccessary in evaluating its survival curve.

Evidence from the study of bacterial genetics indicates the existence of diploid strains of E. coli (Lederberg 1949) and since Latarjet and Ephrussi (1949) have shown that survival curves of haploid and diploid cells of Saccharomyces are respectively exponential and sigmoid, similar differences might be expected with cells of E. coli of different ploidy.

Age of culture. It is not surprising to find that young, growing cells are in general more sensitive to radiation killing than are old, resting cells but differences in the shape as well as in the slope of the curves for such cells would not be anticipated. Roberts and Aldous (1949) however, working with nutrient broth cultures of strain B, assayed by colony count on a mineral agar, found that with young cells an upwardly concave survival curve in which the main component was exponential was obtained and with old cells an upwardly convex or sigmoid curve was obtained. Since the interpretations of radiation effects on microorganisms tend to emphasize the involvement of the genetic apparatus of the cells, it is inter-

esting to consider the possibility that a correlation exists between the relative sensitivity of the young cells and the fact that they can be shown to contain from two to several times as many nuclear bodies (Robinow 1944) as the resting cells. No explicit investigations of this point have been made.

Medium in which the cells are grown. Roberts and Aldous (1949) also include the conditions under which the experimental cells are grown among the factors having an important bearing on their radiation resistance and indicate specifically that cells grown in glucose broth are more sensitive than those grown in broth without added glucose. With X-rays it has been shown conversely (Hollaender, Stapleton and Martin, 1951) that cells of *E. coli* B and B/r are more resistant when grown anaerobically in glucose broth.

Conditions during irradiation. There have been no reports of appreciable changes in the ultraviolet resistance of bacteria as a result of changing the temperature, pH, or the composition of the suspending medium during irradiation. This is also true for changes in the dosage rate. Rentschler, Nagy and Mouromseff (1941) tested the effect of spreading a given dose of ultraviolet over various periods of time and found that the effect was the same for a given dose over a period of time ranging from microseconds to hours.

With ionizing radiations striking changes in the bactericidal action have been shown to occur when the oxygen tension of the suspending medium is changed (Hollaendar, Stapleton and Martin 1951). In general there is a decrease in the effectiveness of a given dose when the amount of oxygen is decreased.

Visible light. In 1949 Kelner reported that an exposure to visible light would greatly increase the survival of spores of Actinomyces griseus which had been irradiated with ultraviolet light. This reversal of the damaging effect of the ultraviolet, now generally called photoreactivation, has been shown to be effective in reducing the killing and mutagenic effect of ultraviolet on Escherichia coli (Kelner 1949 and Novick and Szilard 1949). Among other studies of photoreactivation its general biological signifigance has been indicated by its effectiveness in reducing ultraviolet killing or inhibition of division in bacteriophage (Dulbecco 1950), Arbacia (Blum et al 1950), and Paramecium (Kimball and Gaither 1950).

Photoreactivation of E. coli B has the same quantitative effect seen with most of the factors which increase the resistance of this organism. The survival curve changes from one with an upward concavity to one with an upward convexity. With B/r a quantitative statement of the effect can be made (Kelner 1949 and Novick and Szilard 1949). Here a simple numerical relationship is found between the dose of ultraviolet required to give a particular survival without photoreactivation and the dose required for the same survival with photoreactivation. This factor is the same for every survival point on the two curves.

Liquid recovery. Roberts and Aldous (1948) have shown that an increased survival of E. coli B can be obtained if the irradiated cells are simply held in liquid suspension for a short time before they are plated on solid media. This reaction was shown to

be temperature dependent with recovery occurring at a very slow rate at 5° C. and at a rapid rate at 37° C. The survival curve again changed from one which represented largely exponential killing to one which was essentially sigmoid. The authors discuss this effect in terms of the loss of ultraviolet-produced poisons by diffusion from the cells. B/r does not show this effect.

Medium on which the survival is determined. When survival is determined by colony counts from an irradiated sample, the composition of the plating medium plays a part in determining the number of colonies which develop. Roberts and Aldous (1948) found that an increased survival of strain B was obtained when irradiated samples were plated on a mineral agar medium instead of a peptone, beef extract agar.

Temperature of incubation. The temperature of incubation of the plates on which colony counts are made is important in determining the number of survivors among irradiated cells of strain B. It is surprising that this effect, which can be a very large one, should have gone unnoticed for many years. Anderson (1951) has shown that a large increase in the survival of strain B is obtained when the temperature of incubation is raised from 30° C. to 37° C.

This brief account of the variability of the ultraviolet sensitivity of *E. coli* B shows that the radiosensitive mechanisms in this organism can be characterized by a number of descriptive properties. The purpose of this paper is to present evidence for some additional factors which modify the ultraviolet sensitivity of this organism and to attempt to show some basic similarities in the way

in which these changes are reflected by changes in the shape and slope of the survival curves.

MATERIAL AND METHODS

The stock of *E. coli* B used in these experiments was obtained through Dr. R. L. Dobson from Dr. Evelyn Witkin. Stock cultures for reference were kept on nutrient agar slants at 5° C. and in the dry state in vacuum sealed ampules also stored at 5° C. Daily working cultures were usually prepared by sub-cultivation of the stock of the preceding day. Cultures were prepared in various ways as will be specified but the media were usually based on nutrient broth (Difco) or on the synthetic medium described by Anderson (1946) as follows.

NH_4Cl	0.1%
Na_2HPO_4	0.6
KH_2PO_4	0.3
$MgSO_4$	0.02
NaCl	0.05
Glucose (or other energy source	0.5

Cultures were incubated at 37° C. in a water bath and were aerated during growth by a bubbling stream of air. The incubation time was 16 to 18 hours. With the usual inoculum such cultures were then some 6 to 8 hours past the end of the logarithmic phase of growth and, depending upon the medium, had a titer of about

2×10^9 viable cells per ml. Samples to be irradiated were removed, diluted appropriately and exposed to the ultraviolet light in 1.2 ml amounts spread within a paraffin ring 5 cm in diameter on the surface of a glass plate. Shielding effects were avoided by the use of thin layers of relatively dilute suspensions. The highest cell concentration used was 1×10^8 cells per ml and it was found that screening effects did not appear until the concentration approached 1×10^9 per ml. The depth of the suspension averaged 0.6 mm and it was found that this could be increased to 3 mm without effect. Dilutions were planned in such a way that 0.1 ml samples could be plated directly from the irradiated pools without further dilution. No difference was found in the survival curves from experiments using this method and those in which the samples all contained the same concentration of cells at the time of irradiation and were subsequently diluted for plating.

Survival was determined in all cases by colony counts. The 0.1 ml samples from the irradiated pools were spread on the surface of nutrient agar plates (Difco) with a bent glass rod and the plates then incubated as specified in the results. The count per plate was usually in the range from 100 to 300 and the number of plates prepared for each sample varied. The actual counts obtained are included in the tabulated data. The incubator was controlled by a mercury thermostat and was equipped with a blower. Temperatures were constant to 0.2° C. The time of incubation varied but in each case sufficient time was allowed for detection of all of the colonies which could appear. When lower temperatures were used, this time

was always at least 48 hours.

The ultraviolet source was a low pressure mercury vapor germicidal lamp with its predominant ultraviolet emission in the 2537 Å band. The visible light source was a 1,000 watt, water-cooled, high pressure mercury arc lamp (G. E. AH-6) with some 80% of its total output in the 5400 Å to 6050 Å range. No absolute calibration or spectral characterization was made on either of these sources. The intensity of each source was monitored in a manner sufficiently accurate to give reproducible results. Samples to be exposed to the visible light were held in tubes in a water bath where they were protected from most or all of the ultraviolet radiation by a 6 mm thickness of glass and a 10 mm thickness of water.

RESULTS AND DISCUSSION

The effect of the medium in which cells are grown. The results of a number of determinations of the survival curve for strain B are shown in Figure 1 (Table 1, Appendix I). The culture was prepared in synthetic medium containing 0.5% glucose and the plates were incubated at 30° C. This curve is typical of the two-component, upwardly concave curves obtained by most investigators with this organism. The reason for the upward concavity or "break" in the curve is a matter of interest and no proof of any of a number of possible explanations has been presented in previous work. The most

simple theoretical reproduction of the curve is obtained by plotting the curve that would be expected from a mixture of two kinds of cells each fraction being killed in an exponential manner but at a different rate from the other. The curve of figure 1 can be obtained in this manner by plotting the result of a theoretical situation in which 94% of the population dies according to curve S of figure 1 and 6% according to curve R.

Either an actual difference in the cells or an apparent difference might appear at a number of places in the course of an experiment.

(1) Two kinds of cells differing in their resistance might be present in the experimental suspension. A permanent, hereditary difference could be involved as the result of a mutation to relative resistance or sensitivity or temporary differences might arise in cells which developed under different physiological conditions in an aging culture where the cessation of logarithmic growth might, for example find some cells in a particular stage of division where they would be particularly resistant.

(2) An apparent resistant fraction would be seen if a certain proportion of the cells were shielded from some of the radiation as they would be in the lower layers of a thick or heavy suspension which was not stirred.

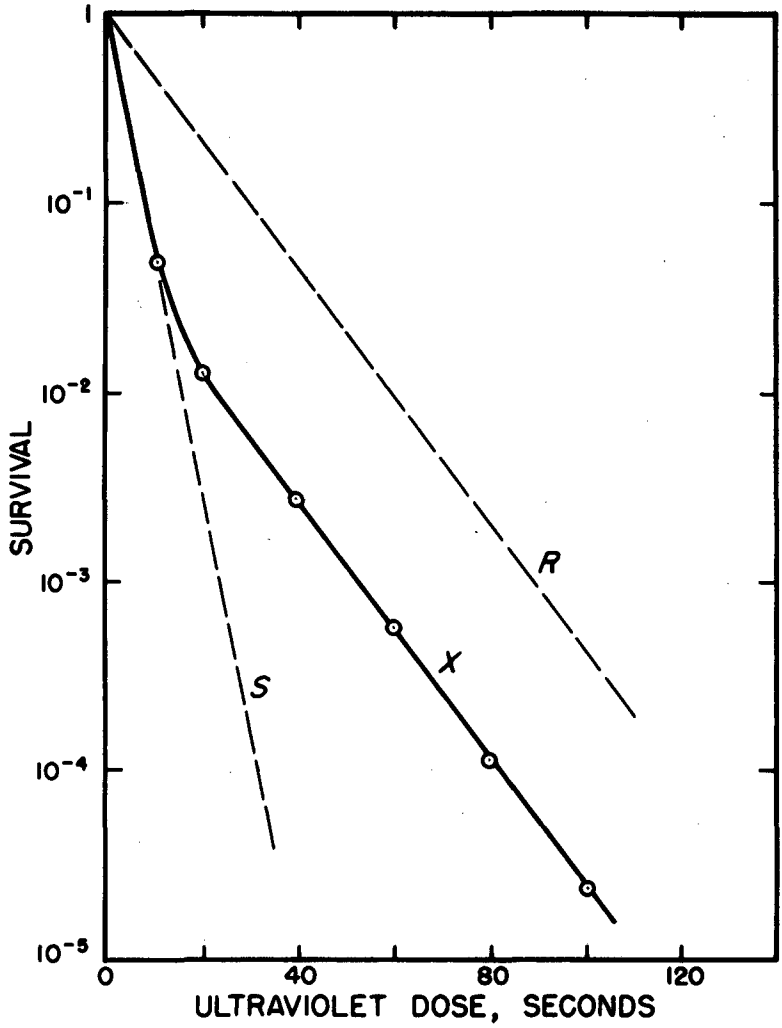
(3) An apparent fraction of resistant cells would also be seen in cases where the post-irradiation treatment allowed a certain proportion of the cells to recover from the damage.

The mutation hypothesis was at least partially rejected by Witkin (1947) who found that the survivors of doses of ultraviolet light which would have left only resistant cells alive were not mutants of the B/r type. The consistency with which the "break" occurs at the same place in a large series of curves also argues against the mutation explanation since large fluctuations in the size of the clone of resistant cells should result when the mutants arise at different times during the growth of the culture (Luria and Delbrück 1943).

The shielding explanation is eliminated in the experiments reported here by the use of thin layers of dilute cell suspensions.

We are left with the possibilities that the difference in resistance is either a temporary one imposed by changes in the conditions under which the cells develop, or a difference which develops during the post-iradiation manipulation, as by the recovery of a certain proportion of the cells of a population initially homogeneous with respect to sensitivity to ultraviolet.

To test the first of these possibilities a number of changes were made in the composition of the synthetic medium. Changes in the concentrations of the various ingredients, the initial pH of the medium, the size of the inoculum, and the temperature of incubation were all without effect in changing the position of the "break" in the survival curve. Cultures were also prepared in nutrient broth and the average curve obtained with cells grown in this medium is shown in Figure 2 (Table 2, Appendix I). There is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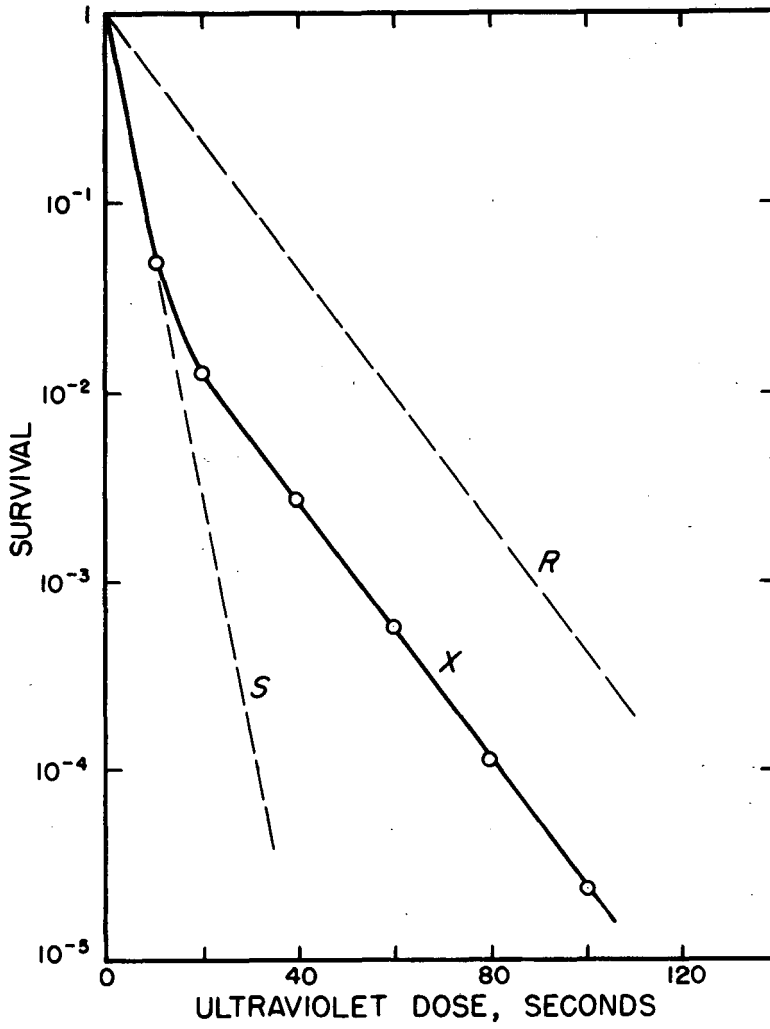
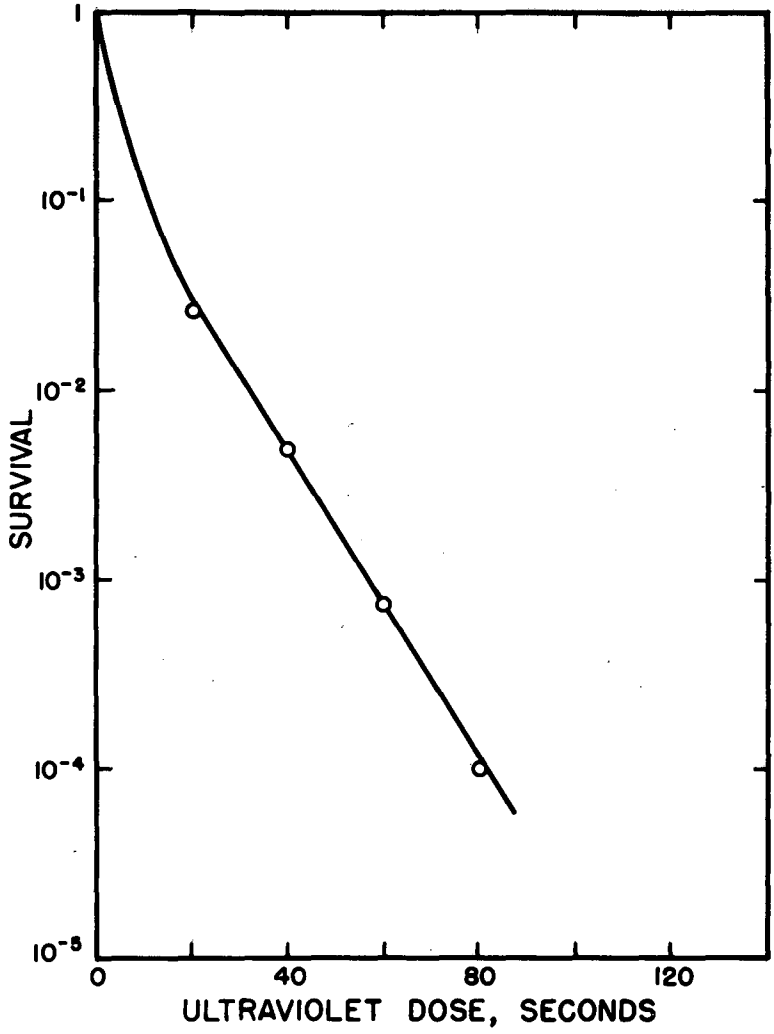


Figure 1. Ultraviolet survival curve of E. coli B, grown in synthetic medium, plated on nutrient agar, and incubated at 30° C. R and S are the theoretical components of this curve.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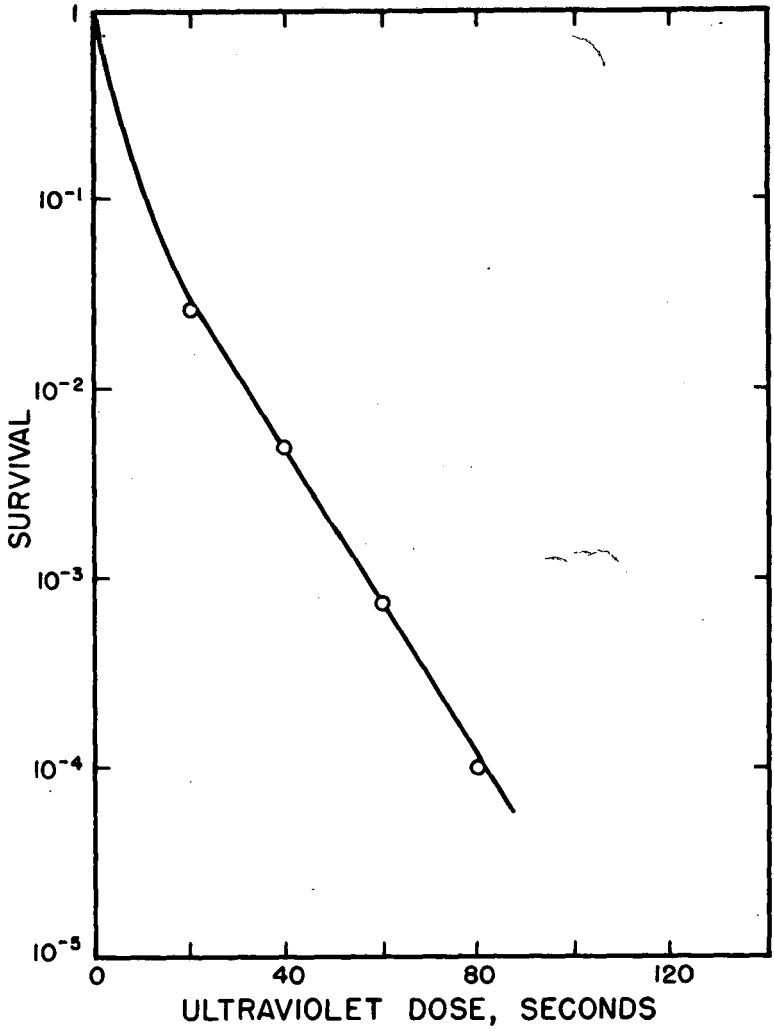
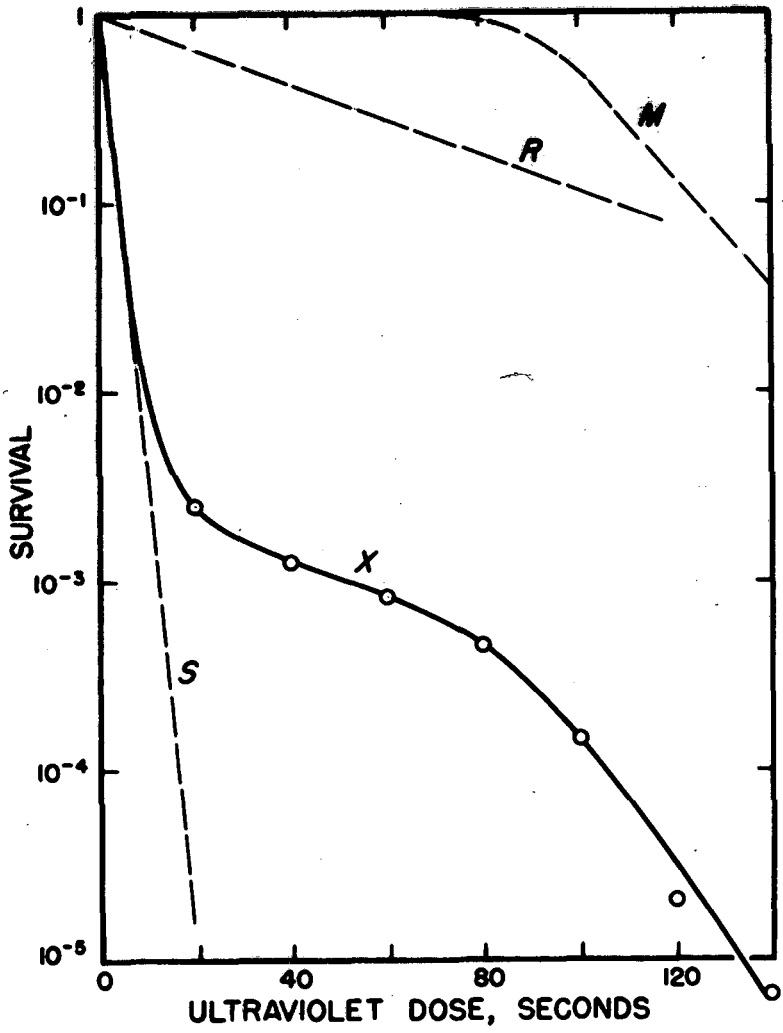


Figure 2. Ultraviolet survival curve of E. coli B, grown in nutrient broth, plated on nutrient agar and incubated at 30° C.

There is no signifigant difference between this curve and that obtained with cells grown in synthetic medium. These results are in good agreement with those of Roberts and Aldous (1948). These investigators reported further, however, that the addition of glucose to the nutrient broth would produce a culture with an increased sensitivity. Figure 3 (Table 3, Appendix I) shows the average of the survival curves obtained in the series of experiments reported here when cultures grown in nutrient broth with 1% glucose were used. This curve differs in several respects from those obtained with cells grown in plain nutrient broth or in synthetic medium. The most striking difference is the introduction of a new component which introduces an upward convexity in the lower part of the curve.

It will be seen that the curve of Figure 3 can be fitted for the most part by means of two exponential curves (S and R) similar to those used for the curve of figure 1. The steeper curve, S, in each case describes the killing which is produced by a relatively efficient radiobiological action. In Figures 1 and 3 the slopes of these components differ only slightly. The more resistant radiosensitive mechanisms, however, now differ in the extent to which they contribute to the total killing. This is reflected in the difference in slope of the R components of figure 1, for cells grown in synthetic medium, and Figure 3, for cells grown in nutrient broth with glucose, In the latter case it is seen that while the sensitivity of the effect described by curve R is decreased, a smaller proportion of cells is involved primarily with it.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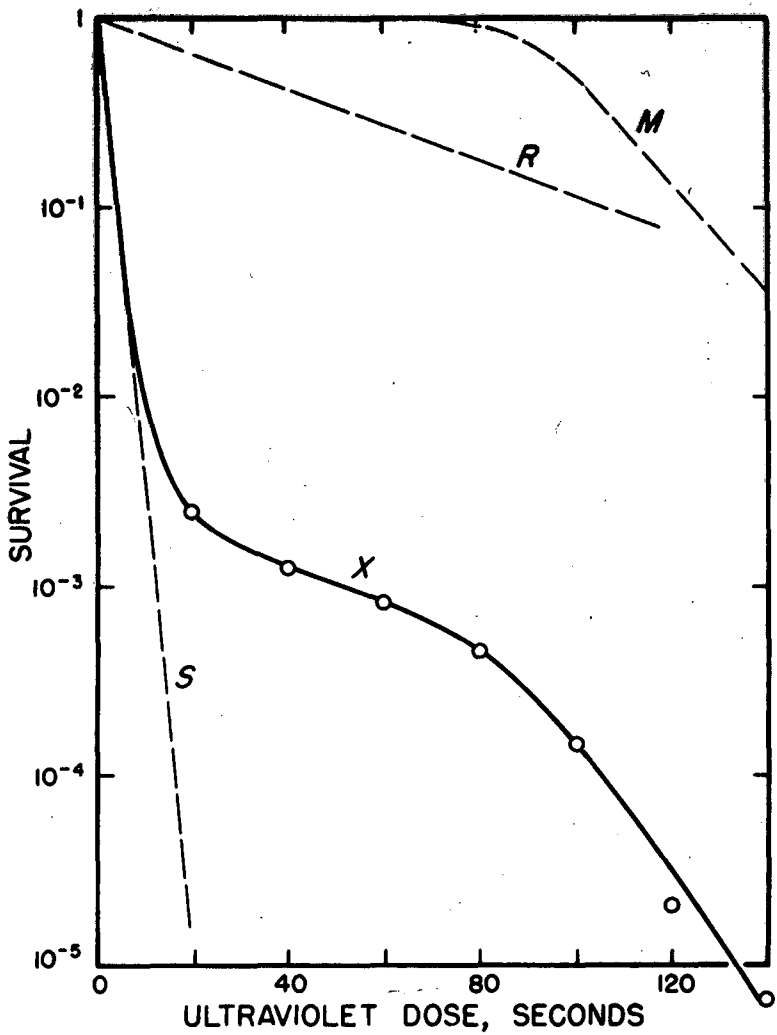


Figure 3. Ultraviolet survival curve of E. coli B, grown in glucose broth, plated on nutrient agar and incubated at 30° C. S, M, and R are the theoretical components of this curve.

In addition, the cells which are killed by the process indicated by this curve R of Figure 3 are also susceptible to being killed by a third mechanism which is described by by the theoretical sigmoid or multiple-hit curve, M, which is also shown. It seems reasonable at this point to make the further simplifying assumption that, in addition to the cells killed by the process represented by curve R, also the cells killed by the process represented by curve S were susceptible to this multiple-hit effect.

The fitting of the experimental survival curves obtained in various experiments by means of appropriate combinations of simple exponential and sigmoid curves has been a useful procedure. Apparently complex differences in the mechanism of ultraviolet killing for cells grown in synthetic medium and those grown in nutrient broth with glucose can be resolved into a few simple changes in the relative effectiveness of a small number of lethal mechanisms. The assumption implied here, that the same mechanisms are involved in each case and that they differ only in their relative importance, will be shown to have considerable justification.

A brief discussion of the theoretical curves used and the ways in which they can be combined is found in Appendix II. When these methods are applied to the curve of Figure 3, the equation for the complete curve will take the following form.

$$S = 0.9965\ e^{-sd} + 0.0035\ e^{-rd} - (1 - e^{-md})^{100}$$

S is the fraction surviving, d is the radiation dose, and s, r, and m are proportionality constants for the theoretical components

S, R, and M respectively. The factors 0.9965 and 0.0035 represent respectively the fraction of the original population dying according to curves SM and RM.

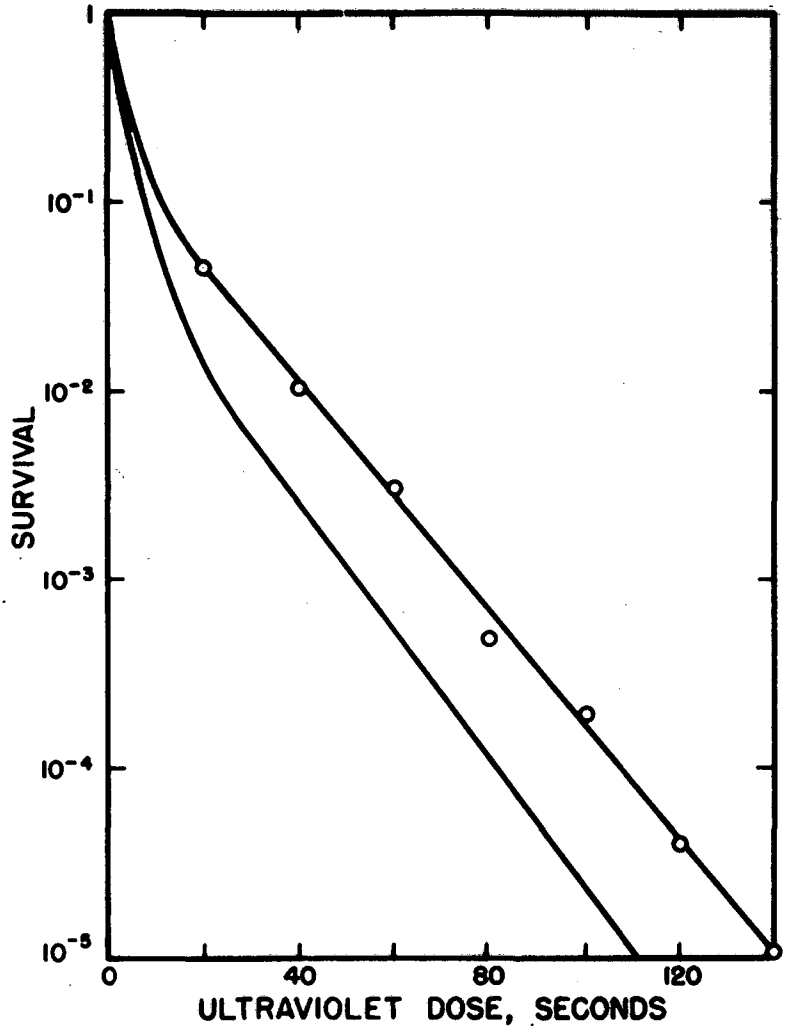
The influence of glucose in the nutrient broth cultures is apparently to provide a fermentable substrate. Both B and B/r carry out a vigorous fermentation of glucose under aerobic or anaerobic (nitrogen bubbled) conditions in glucose broth but the absence of gas production in synthetic medium cultures, even when glucose is provided, and the inability of these organisms to grow anaerobically in glucose, synthetic medium cultures indicates that under these conditions their metabolism is essentially oxidative. This observation of an overall increase in ultraviolet sensitivity of E. coli when grown under conditions where it can carry out a fermentative type of metabolism stands in interesting contrast to the situation which exists with ionizing radiations. Hollaendar, Stapleton and Martin (1951) have shown that cells of B and B/r grown anaerobically in glucose broth or aerobically in the same medium, where they presumably still choose a fermentative type of metabolism, are more resistant to X-rays than cells which have been grown in plain nutrient broth where they are primarily oxidative.

pH of the plating medium. Although the survival curve for B, grown in synthetic medium (Figure 1) was quite reproducible from one culture to another over long periods of time, there would be an occasional period when it would be observed to shift for a number of experiments to the curve shown in Figure 4 (Table 4, Appen-

dix I). The curve of Figure 1 is reproduced here for comparison. The essential difference between these two curves is a difference in the position of the "break" in the curve. Renewed efforts to isolate the unknown variable involved in determining the size of the fraction of apparently resistant cells finally directed suspicion to the pH of the plating medium on which colony counts are made. Figure 5 (Table 5, Appendix I) shows a series of ultraviolet survival curves obtained with cells grown in glucose synthetic medium, irradiated and then plated on sets of nutrient agar plates which had been adjusted to various pH values by the addition of NaOH or HCl to the melted agar before pouring.

Figure 6 (Table 6, Appendix I) shows the same experiment conducted with cells which had been grown in nutrient broth plus glucose. Here the curves on media at pH 7.2 and 8.3 were sufficiently close together to be averaged into one curve as shown. The pH 6.8 curve is reproduced from figure 3. The difference in survival produced by slight pH changes indicates that a shift of the sort shown in Figure 4 might occur at intervals when some unnoticed change was made in the method of preparation of the nutrient agar. At the time that this pH effect was found, for example, the nutrient agar routinely used without adjustment was stated on the manufacturer's label to have a final pH of 6.8 while under our conditions of preparation the final pH was measured as 6.5.

Considerable information can be obtained from the curves of Figure 6 where the effect of changing the pH of the plating medium is most prominent. If the theoretical components S, R and M of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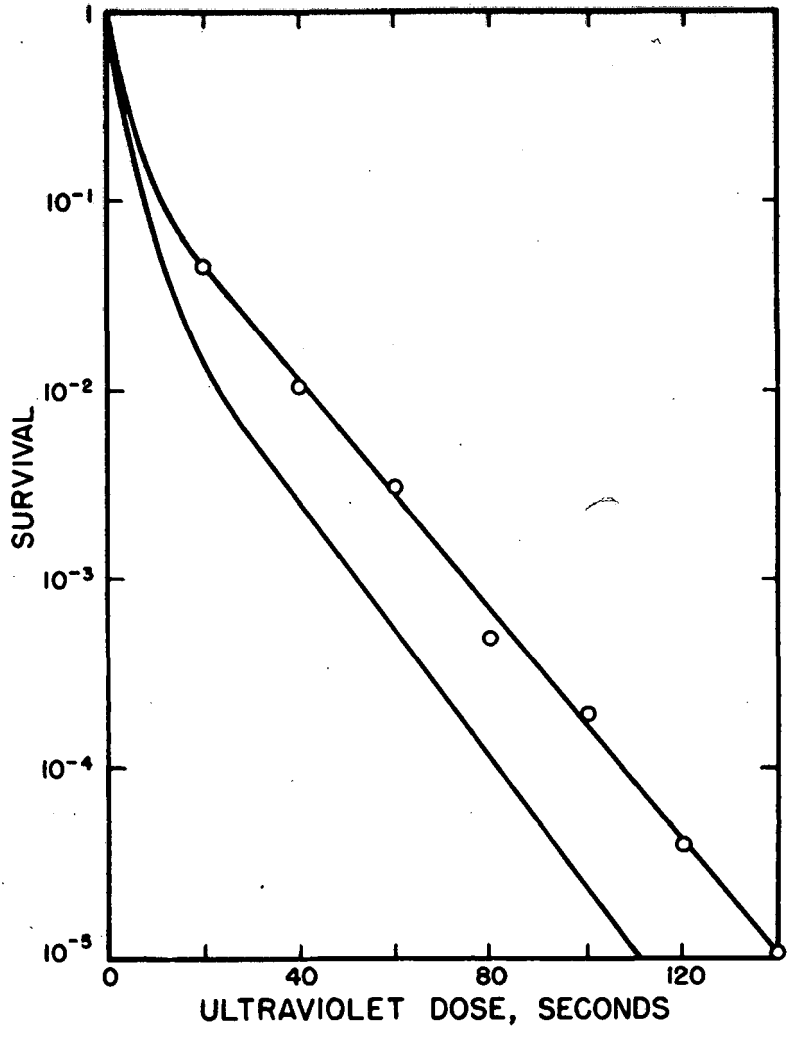
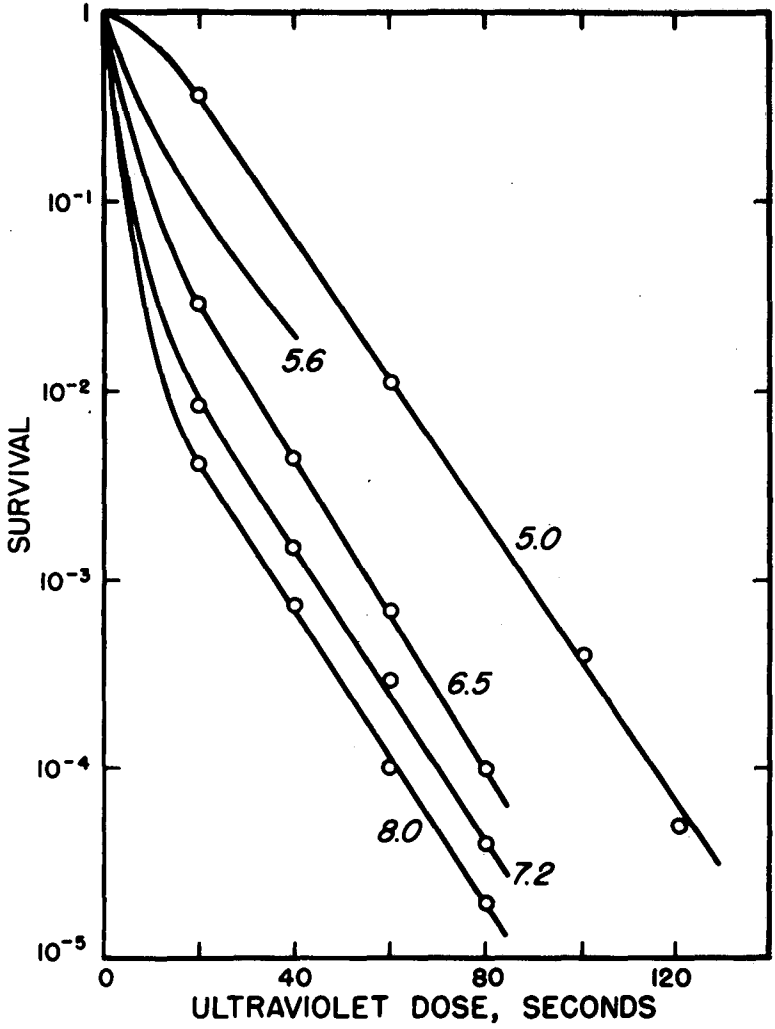


Figure 4. Ultraviolet survival curve of E. coli B, grown in synthetic medium, plated on nutrient agar and incubated at 30° C. The lower curve is reproduced from Figure 1.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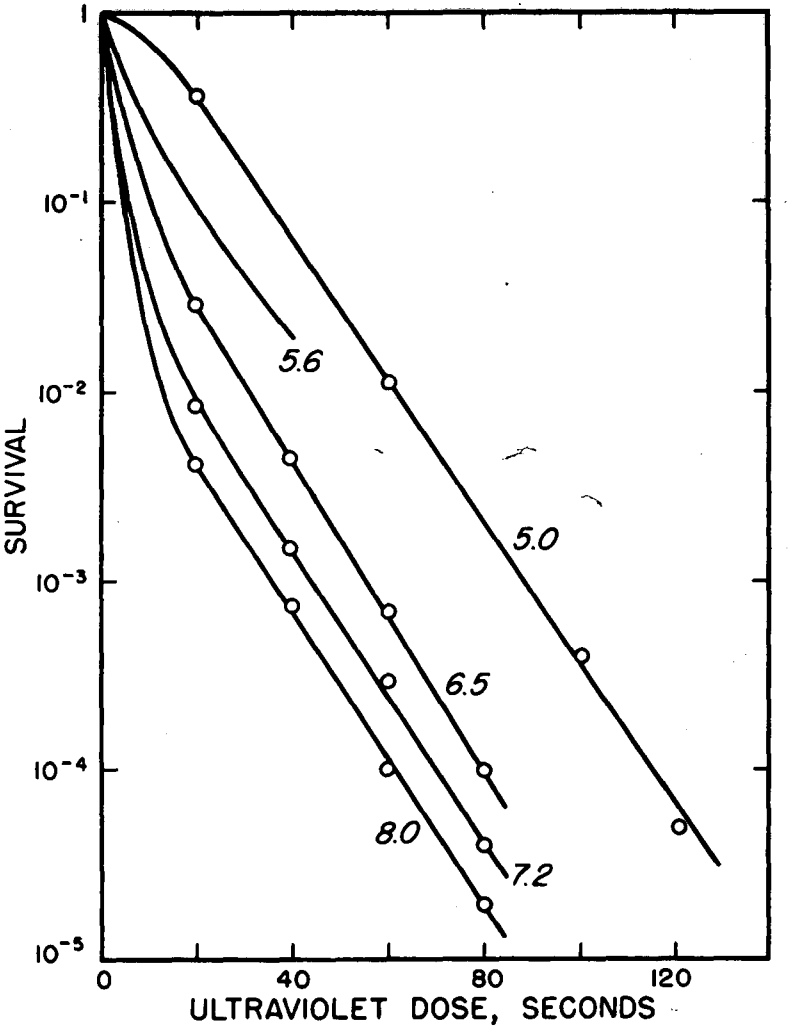
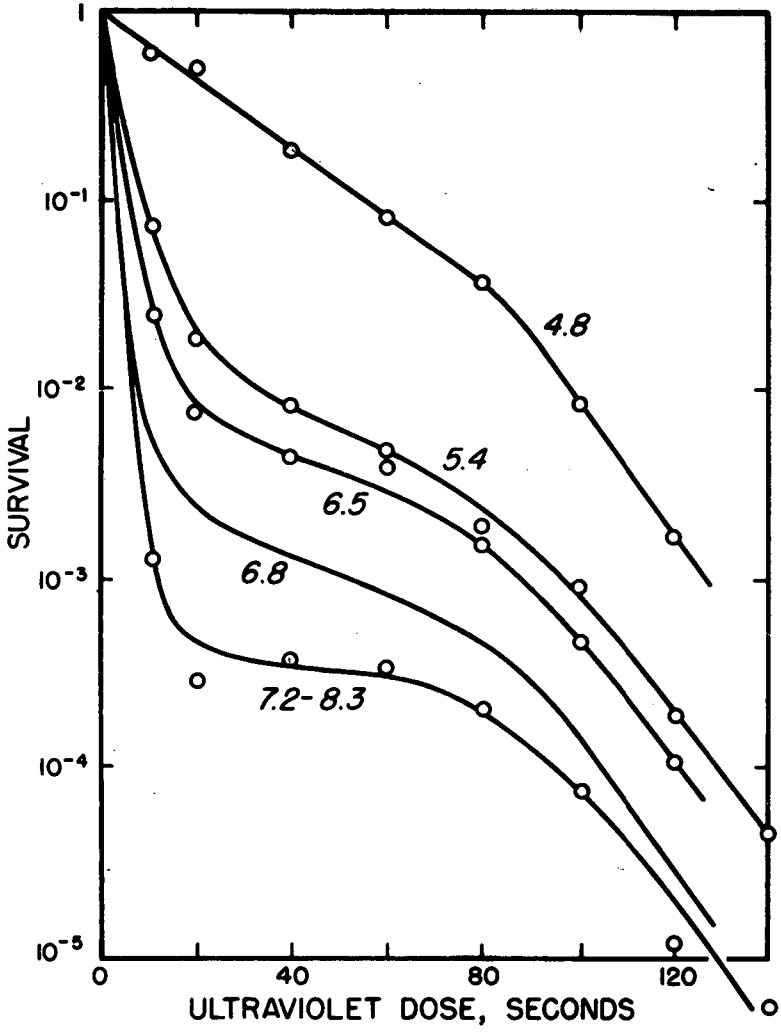


Figure 5. Ultraviolet survival curve of E. coli B, grown in synthetic medium, plated on nutrient agar adjusted to the pH values shown on the curves, and incubated at 30° C.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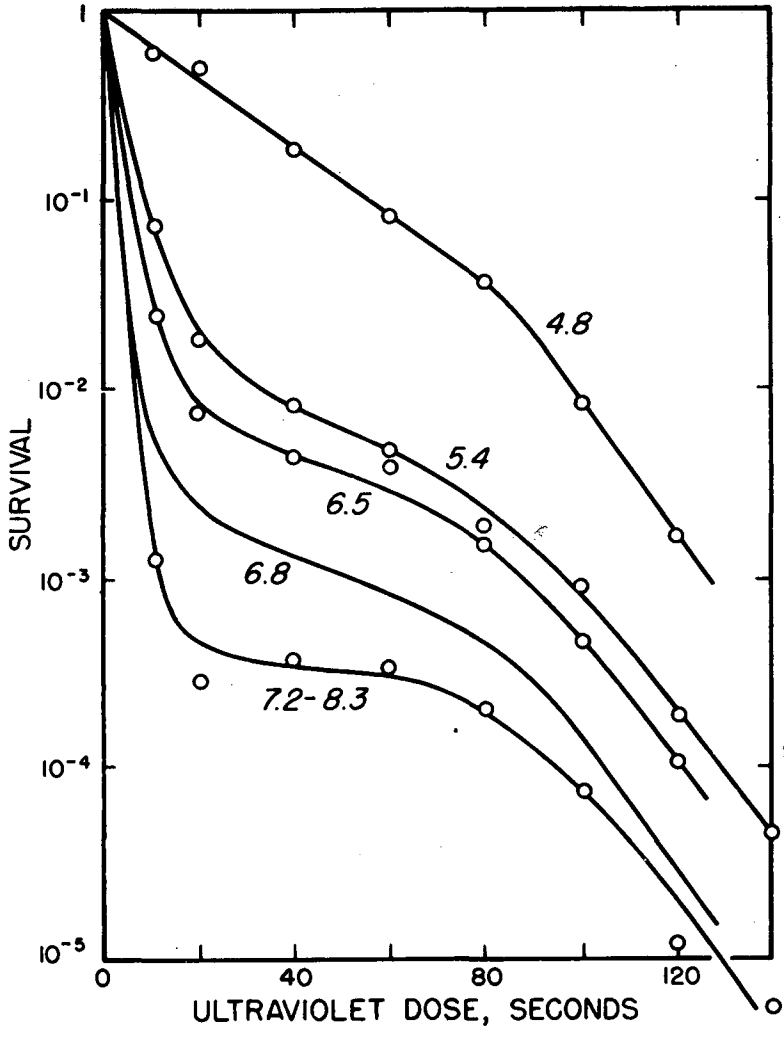


Figure 6. Ultraviolet survival curves of E. coli B, grown in glucose broth, plated on nutrient agar, adjusted to the pH values shown on the curves, and incubated at 30° C.

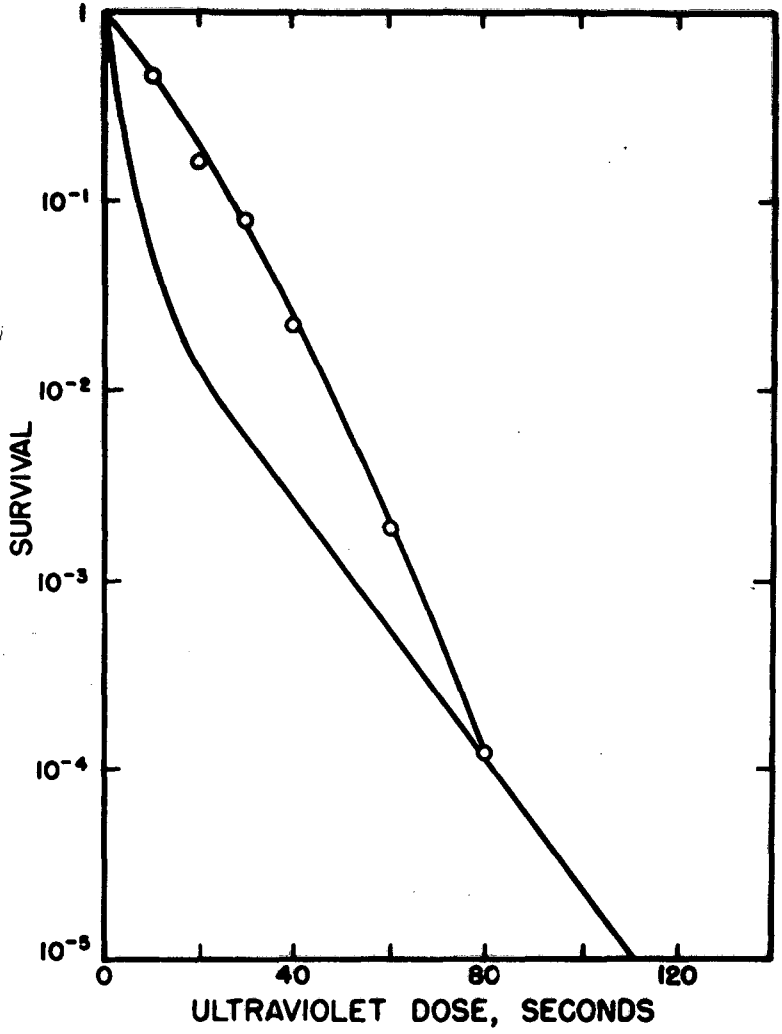
Figure 3 are recalled, it will be seen that a number of changes in their values occur as one changes the pH of the plating medium. First there is approximately a thousand-fold change in the relative proportions of the cells killed by the processes represented by curves S and R. This is shown by the upward shift in the position of the break in the curve. It will be remembered that two possible explanations for this upward concavity have been offered. (1) Two kinds of cells with different sensitivities to ultraviolet are present and (2), all cells are initially equally sensitive but the effect is subsequently removed from some of them. The first of these is no longer tenable because the pH experiments require it to imply that there is a difference in survival of the radiation-resistant and radiation-sensitive portions of the population due to pH alone and this would result either in a thousand-fold increase or a ten thousand-fold decrease in the control count on the extreme acid and extreme alkaline plates respectively. This change is not seen.

Thus it can be concluded with some conviction that the "break" in the survival comes about because some of the cells have an opportunity to recover from the primary radiobiological effect which accounts for most of the killing.

The efficiency of the killing process which results in the survival curve R is also appreciably affected by the pH of the plating medium. The slope of the theoretical component R which can be derived for the various curves of Figure 6 undergoes a sizeable change as the pH is changed.

Effect of visible light. In the course of some work with photoreactivation some experiments were performed to test the effect of an exposure to visible light made before the irradiation with ultraviolet light. In agreement with Kelner (1949) it was found that visible light exposure previous to ultraviolet irradiation had no effect upon the survival of B/r, but it was found that the B strain of *E. coli* could be photodesensitized by such treatment. Figure 7 (Table 7, Appendix I) shows the survival curve obtained with cells from a synthetic medium-glucose culture after a preliminary exposure of five minutes to the light from the AH-6 lamp. The "dark" curve, reproduced from Figure 1, differs only in representing the survival of cells which were not exposed to the visible light. In Figure 8 (Table 8, Appendix I) similar curves are shown for cells grown in glucose broth. The survival curves obtained after exposure to two lesser doses of visible light are also shown.

The main effect of photodesensitization on the radiobiological effect which accounts for the steep exponential portion of the "dark" curves is indicated by a change in the slope of this theoretical curve S in the direction of increased resistance. No tendency is seen here, however, for a change in the ratio of cells affected primarily by either process S or R. In the curves obtained after lower doses of visible light, where the R component, can be detected, it remains unchanged. An analysis of the photodesensitized curves is encumbered by the neccessity of introducing a fourth theoretical component in the form of a multiple-hit curve, to account for the initial upward convexity in the experi-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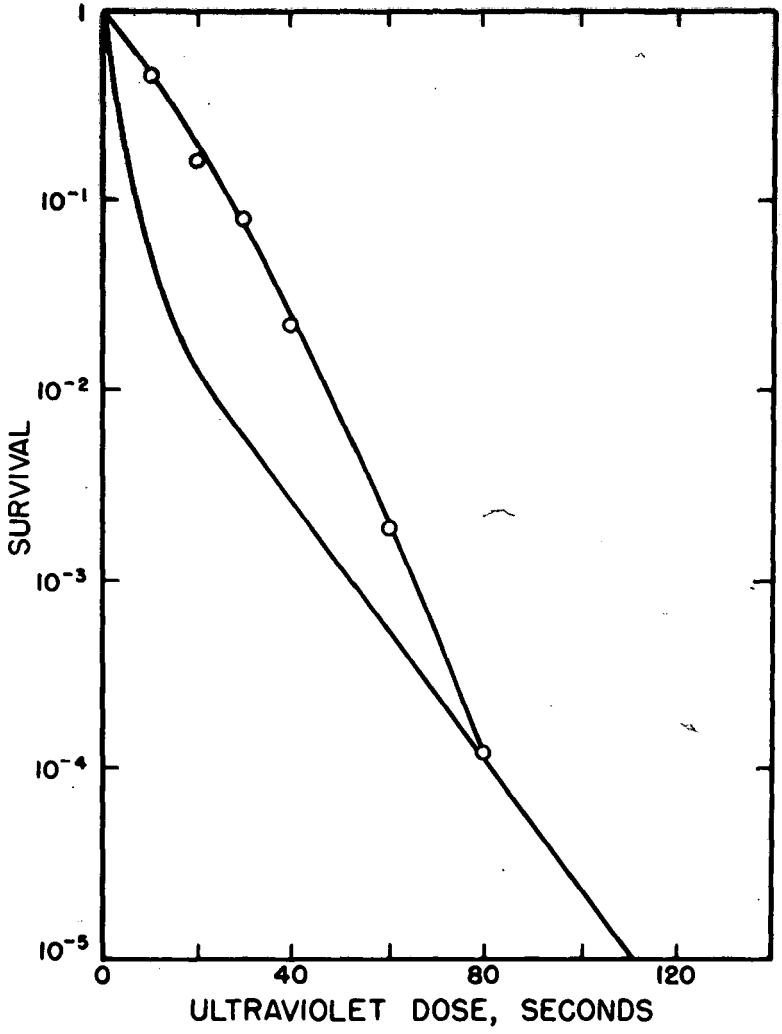
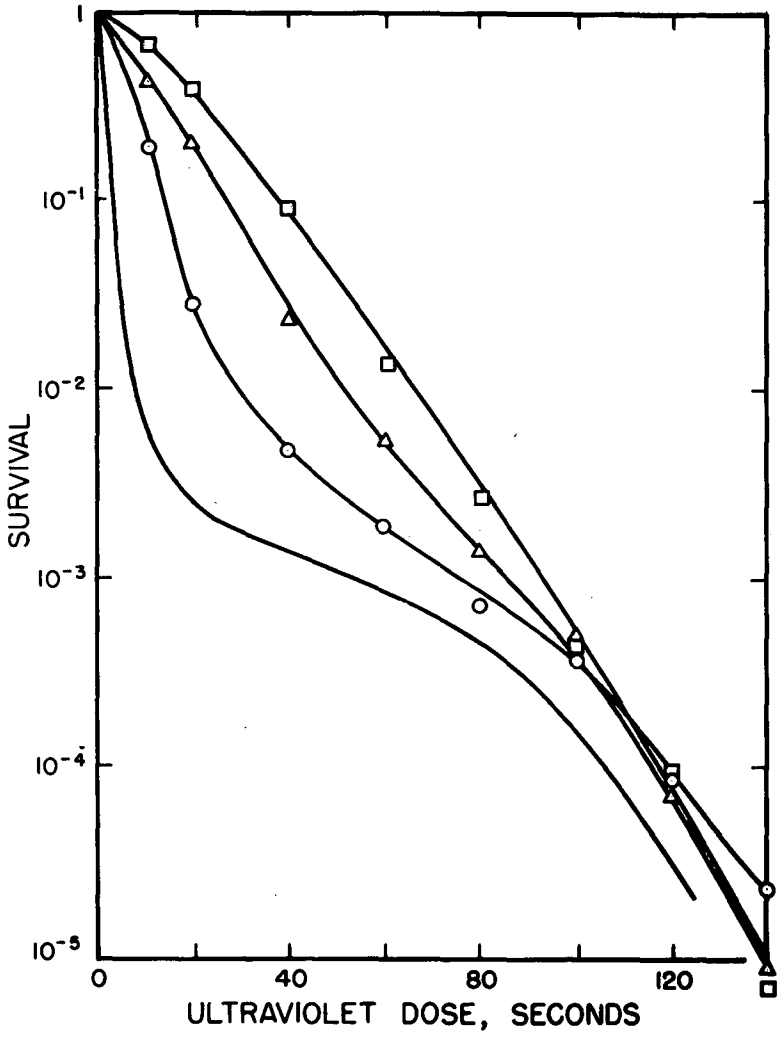


Figure 7. Ultraviolet survival curve of E. coli B, grown in synthetic medium and photodesensitized before irradiation. Incubated at 30° C. The lower curve, from Figure 1, is the same without photodesensitization.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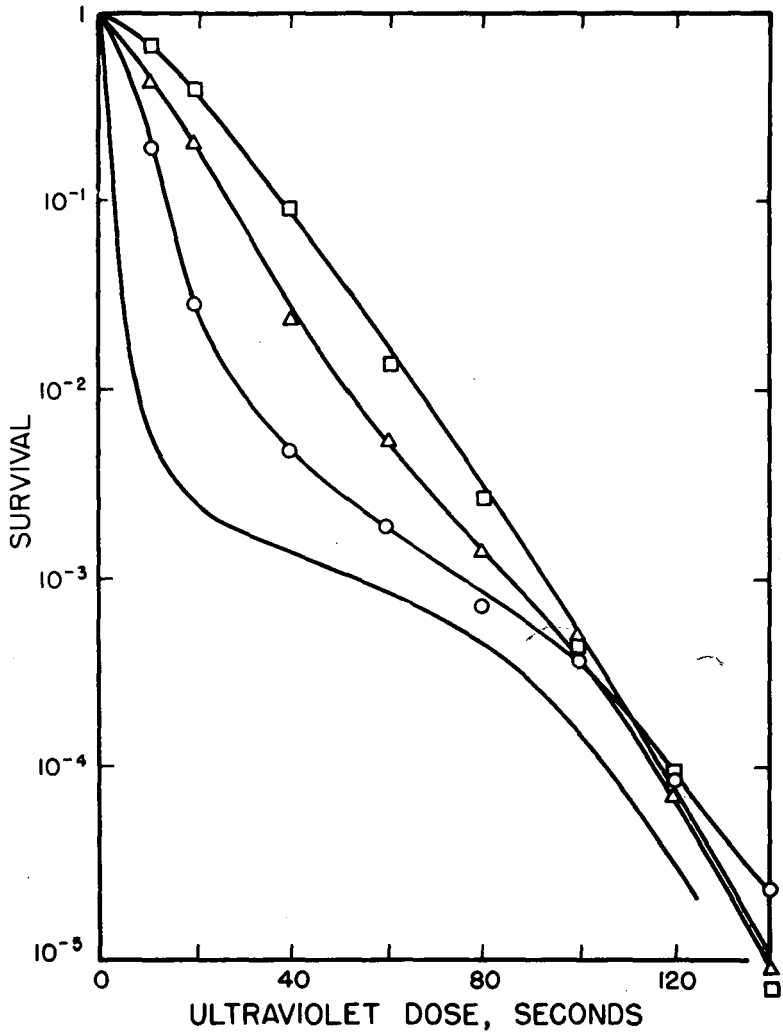


Figure 8. Ultraviolet survival curves of E. coli B, grown in glucose broth and photodesensitized. Visible light exposure for curves, from top to bottom, was 4, 2.5, 1.5 and 0 minutes. The bottom curve is reproduced from Figure 3.

mental curve. It is not clear at present what relation this radiobiological effect has to the other effects under discussion.

It has been shown that, under certain conditions, (Figures 1, 2, 6) that the survival curve of E. coli B is characterized by a very steep component which accounts in some cases (Figure 6) for as much as 99.9 percent of the killing. It has also been seen that the particular effect which this curve describes is a very labile one, capable of losing most or all of its effectiveness when the various factors which have been described are applied. We are now in a position to say a great deal about this particular radiobiological effect in this particular organism.

(1) The effect is represented by an exponential dose-effect curve and thus, subject to the reservations indicated by Lea (1946) for such an interpretation, it is a one-hit effect, initiated by the absorption of a single photon of 2537 Å ultraviolet.

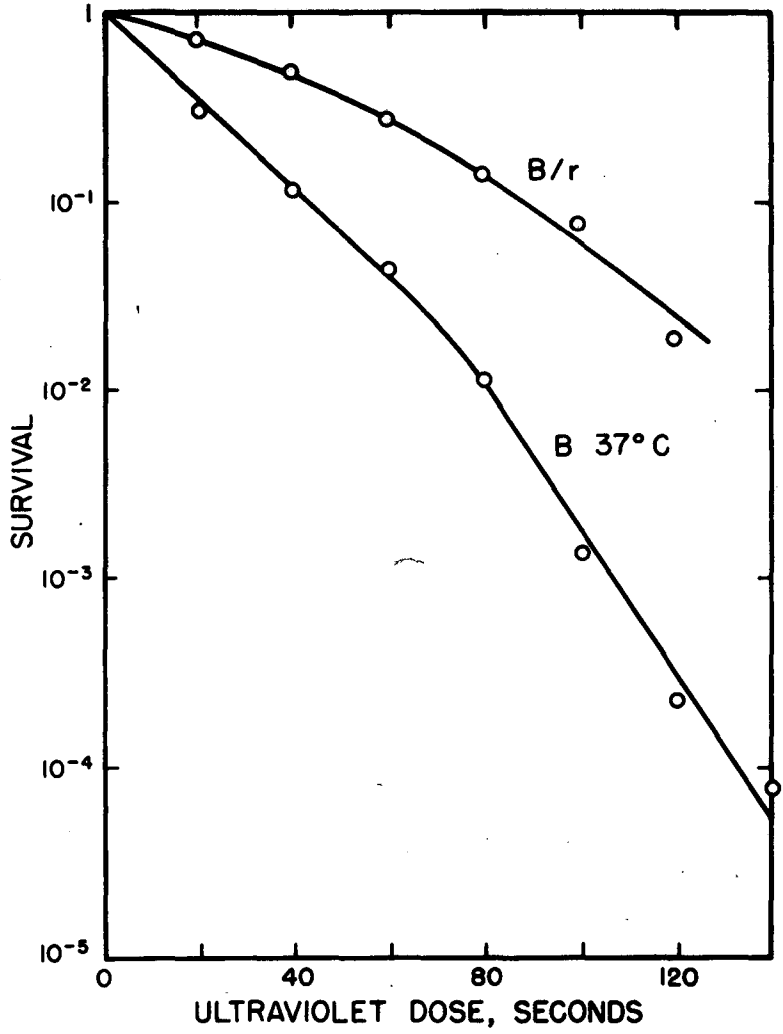
(2) The various ways in which recovery can be effected indicate that the absorption of this photon is temporally and, it seems probable, spatially separated from the final, irreversible process which determines that the cell will not be able to give rise to a visible colony.

(3) Because of the very small chemical change produced by the excitation energy of a single photon, the chemical configuration of the absorbing structure must be highly specific in its importance to the development of the cell. Also, since one such changed structure is sufficient to kill the cell, it must be involved in some unique and indispensable reaction in the cell.

(4) The ultraviolet survival curve of the radiation-resistant mutant, B/r, is shown in Figure 9 (Table 9, Appendix I). This curve can be fitted, as was the lower part of the curve of figure 3, by assuming that each cell is susceptible to being killed in one of two ways, one represented by an exponential curve and the other by a sigmoid curve. The very steep exponential component seen in the survival curves of strain B is no longer evident in the curves for the resistant mutant. Thus the particular effect under discussion is seen, on the basis of Witkin's evidence (1947), to be dependent upon the presence of a single gene.

(5) Figure 3 and the accompanying discussion show that the lethal action of the ultraviolet is most effective on cultures grown under conditions where the cells engage primarily in a fermentative metabolism. In such cultures it has also been shown that there is less chance that a certain number of the cells will recover from the effects of the radiation.

(6) The curves of Figure 5 and, more particularly, Figure 6, showing the effect of changing the pH of the plating medium, have as their most striking difference a change in the size of the apparent resistant fraction. The nature of these curves indicates that the reason for the "break" in the curve is found in the fact some of the irradiated cells are able to recover from the main radiobiological effect which accounts for most of the killing. When nutrient agar of pH 4.8 is used instead of agar at pH 8.3, for example, an approximately five thousand-fold increase in the number of apparently resistant cells is seen. Since the control


1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
SURVIVAL
B/r
B 37° C
0
40
80
120
ULTRAVIOLET DOSE, SECONDS

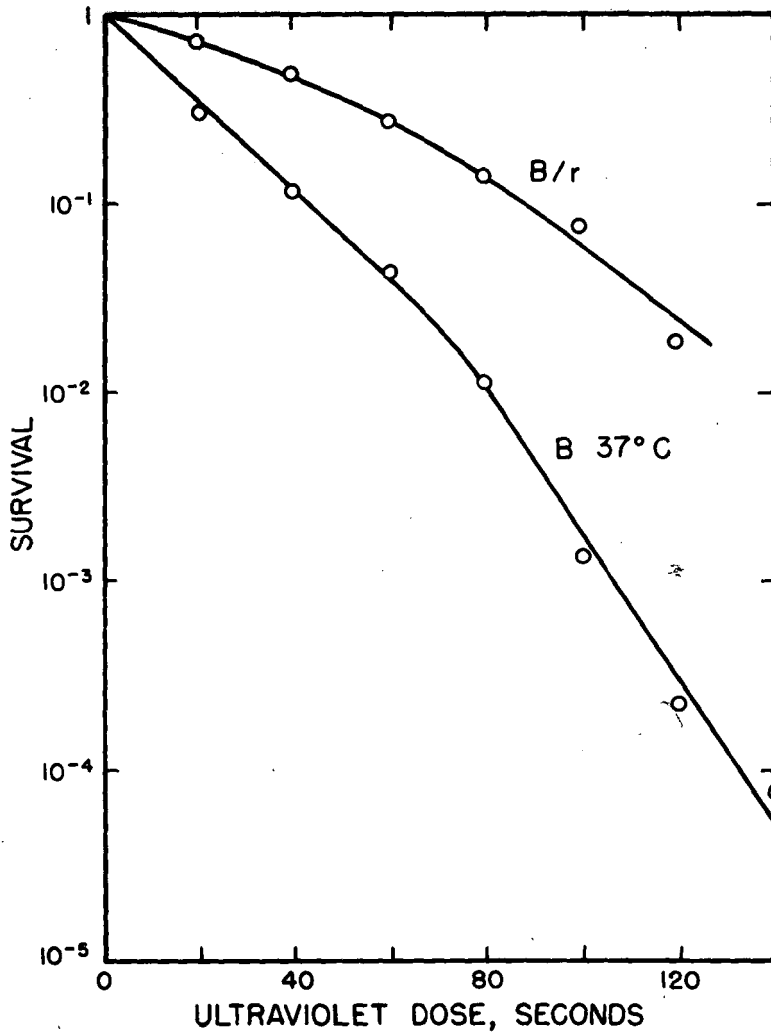


Figure 9. Ultraviolet survival curve of E. coli B/r (upper curve), incubated at 30° C. The lower curve is for strain B, grown in synthetic medium and incubated at 37-40° C.

count in this instance changes at most by a factor of two, it cannot be argued that the pH effect represents only a relative increase due to an increased survival of resistant cells in the population, or a decreased survival of sensitive cells, due to the pH change alone. It is concluded that an all-or-none change from relative sensitivity to relative resistance can occur in the irradiated cells and that the efficiency of this change depends upon the pH of the plating medium.

(7) The survival curves obtained after photodesensitization with visible light (Figures 7 and 8) show that a further characteristic of the effect under discussion is that its efficiency can be impaired by exposing the cells to visible light previous to the irradiation with ultraviolet.

(8) The temperature of incubation of the plated samples was mentioned in the introduction as another factor that has been shown to influence the number of survivors. Figure 9 contains a survival curve of strain B, grown in synthetic medium, irradiated, and plated at 37° C.

At this point it is not difficult to set up a model, consisting of a series of hypothetical structures and events, which will account for all of the effects reported here. The value of such a model depends, of course, upon its fruitfullness in suggesting new experiments which will narrow the, at present, wide gap between the hypothetical pictures which have been proposed for various radiobiological effects and the specifically known structures and functions of the cell. We should like to propose the following as a

tentative model which has proved useful in the present work.

When one considers the list of factors which have been shown to influence the susceptibility of E. coli to the lethal effect of ultraviolet radiation, the impression is gained that their importance lied in their ability to determine the efficiency with which some active agent mediates the chain of events between the absorption of the radiant energy and the final chemical change which determines that the cell will not divide. This then implies, for want of an alternative if for no other reason, that one or more enzymes, or enzyme systems, are responsible for transferring some constituent of the cell from the site where it has been chemically altered by the radiant energy, to some critical locus where its effect is now that of a poison. The model is simplified if it can be further assumed that the system is one of a number of alternative pathways and that its presence is not indispensable to the life of the cell. If such were the case, the mutation from strain B to B/r could be viewed as a familiar loss mutation and the factors of temperature, pH and visible light can be readily pictured as interfering with the efficiency of the poison transfer without otherwise affecting the normal growth of the cell. When this system has been temporarily blocked, the poison can decompose, be removed to some non-critical structure, or be outnumbered by non-poisonous competitors. The difference in sensitivity between aerobically and anaerobically (fermentative) grown cells suggests that study of the known biochemical differences in such cells might offer suggestions as to the identity of the systems involved.

SUMMARY

The ultraviolet survival curve of aerobically grown cells of *Escherichia coli* B has an initial steep exponential component which eventually becomes upwardly concave and then extends into a second, less steep, exponential component. The radiobiological effect which accounts for the first portion of this curve, and which accounts in the usual case for almost all of the killing, is particularly susceptible to being reduced in its effectiveness by a variety of changes in the physical and chemical conditions of an experiment.

With cultures prepared under conditions where a fermentative type of metabolism takes place the cells show an increased sensitivity to the primary effect described above. The shape of the survival curve also indicates that, in this case, a third radiation effect is contributing to the total killing.

An increased resistance to ultraviolet light is shown to result when the irradiation is preceded by an exposure to visible light. This effect, which is called photodesensitization, is not obtained with the radiation resistant mutant, B/r, of *E. coli.* The survival curves obtained after various doses of desensitizing light suggest that the effect of the visible light exposure is to reduce the effectiveness of the ultraviolet in killing by means of the particularly sensitive mechanism under discussion.

The survival of irradiated cells is also shown to be strongly dependent upon the pH of the plating medium on which colony counts

are made. From the survival curves obtained on media adjusted to various values of pH it is concluded that the pH is important in determining the probability that an irradiated cell will recover from the primary killing effect. These curves also afford an opportunity to state that the upward concavity which is found in the usual curve is due to the fact that a certain proportion of the irradiated population has been able to recover from the radiobiological effect, and not to the fact that the population was inhomogeneous with respect to sensitivity to radiation.

ACKNOWLEDGEMENTS

This work was made possible by the material assistance and advice of Dr. R.L. Dobson of the Division of Medical Physics of the Radiation Laboratory of the University of California. Thanks are due to Dr. Dobson and to other members of the staff of the Donner Laboratory for help and discussion and to Drs. R.Y. Stanier and M. Doudoroff of the Dpartment of Bacteriology who have acted as Graduate Advisors for this work.

APPENDIX I

Tabular data for the figures.

Table 1.

Seconds exposure to u.v.

Exp.		0	10	20	40	60	80	100
423	Count	210	160	143	196	220	195	103
	% S		3.1	.68	.28	.063	.0093	.0015
404	Count	502		495	121	100	343	58
	% S			.99	.23	.020	.0068	.0012
404	Count	452		340	89	176	602	259
	% S			.75	.19	.039	.013	.0057
404	Count	473		599	114	157	75	225
	% S			1.3	.23	.033	.016	.0047
403	Count	552	113	252	97	277	21	49
	% S		2.1	.47	.18	.050	.005	.0017
402	Count	305	194	406	106	189	291	36
	% S		6.4	1.3	.35	.062	.0095	.0012
401	Count	367	203	478	128	352	93	131
	% S		5.4	1.3	.34	.094	.025	.0035
401	Count	740	294	539	180	490	150	332
	% S		4.0	.73	.24	.066	.020	.0045
510	Count	365		316	338	575	560	
	% S			.80	.17	.044	.0071	
395	Count	1216		436	442	306	276	
	% S			1.4	.36	.10	.023	
396	Count	760		442	413	251	200	
	% S			1.5	.46	.092	.018	
431	Count	412		730	556	464	341	103
	% S			1.9	.36	.061	.0074	.00075
433	Count	758		1563	980	722	774	290
	% S			2.2	.35	.052	.0092	.0012
418	Count	690	1069	731	294	155	103	246
	% S		.77	1.1	.13	.022	.0030	.0010

Table 1 (continued)

		Seconds exposure to u.v.						
Exp.		0	10	20	40	60	80	100
419	Count	612		1200	295	373	65	
	% S			2.0	.38	.061	.011	
438	Count	518		877	620	625	552	330
	% S			1.8	.32	.065	.0096	.0019
Average % S			4.9	1.3	.28	.058	.012	.0023
Average deviation			1.7	.41	.079	.018	.0053	.0014

Table 2

		Seconds exposure to u.v.				
Exp.		0	20	40	60	80
471	Count	331	869	992	442	344
	% S		5.2	.90	.13	.021
487	Count	215	241	383	485	748
	% S		2.5	.40	.051	.0079
489	Count	240	337	461	554	693
	% S		2.8	.38	.046	.0058
494	Count	417	295	383	375	662
	% S		2.1	.37	.045	.0095
494	Count	417	284	428	497	708
	% S		2.4	.41	.060	.010
499	Count	358	210	221	232	266
	% S		2.3	.37	.052	.0075
517	Count	266	418	412	449	
	% S		4.7	.93	.17	
409	Count	595	939	217	319	61
	% S		1.6	.37	.054	.010
409	Count	594	630	159	282	21
			1.1	.27	.048	.0035
Average % S			2.7	.50	.074	.010
Average deviation			.93	.19	.04	.0039

Table 3

Exp.		Seconds exposure to u.v. 0	20	40	60	80	100	120	140
587	Count	84		207	155	76	17		
	% S			.12	.092	.045	.010		
582	Count	354	585	325	274	261	98	115	31
	% S		.17	.092	.077	.037	.014	.0033	.00088
568	Count	435	90	173	241	411	1012		
	% S		.12	.080	.055	.047	.023		
596	Count	806	2200	834	426	240	507	405	82
	% S		.55	.21	.11	.060	.013	.0025	.00051
Average % S			.26	.13	.084	.047	.015	.0021	.00069

Table 4

Seconds exposure to U. v.

Exp.		0	20	40	60	80	100	120	140
528	Count	175	137	452	498				
	% S		3.9	1.3	.29				
557	Count	135	274	197	208	139	93	59	
	% S		5.1	.73	.14	.021	.0028	.00035	
530	Count	371	506	453	244				
	% S		5.5	.98	.13				
530	Count	345	410	421	313				
	% S		4.8	.98	.18				
533	Count	806	691	784	924				
	% S		4.2	.97	.23				
543	Count	643	748	837	2400		3300	2800	525
	% S		5.8	1.3	.75		.041	.0087	.0016
546	Count	666	157	160	208	269	274	201	298
	% S		4.7	.96	.31	.078	.017	.003	.0006
547	Count	423	618	627	561				
	% S		5.2	1.2	.22				
531	Count	341	395	585	847				
	% S		5.8	1.7	.50				
531	Count	356	291	510	816				
	% S		4.3	1.4	.46				
549	Count	164	102	172	284				
	% S		3.1	1.1	.35				
549	Count	157	96	137	219				
			3.1	.87	.28				
Average % S			4.6	1.1	.32	.050	.020	.0040	.0011
Av. deviation			.76	.17	.13	.029	.010	.0031	.0005

Table 5.

Exp.		Seconds exposure to u.v. 0	20	40	60	80	100	120
pH 5.0								
557	Count	120	180		145		129	76
	% S		38		1.2		.043	.0050
pH 5.6								
553	Count	181	357	403				
	% S		9.4	2.1				
pH 6.5								
556	Count	575	664	529	543	456		
	% S		2.9	.46	.082	.014		
pH 7.2								
556	Count	514	175	161	157	111		
	% S		.86	.16	.031	.0043		
pH 8.0								
556	Count	498	167	96	51	50		
			.42	.096	.010	.0020		

Table 6.

Seconds exposure to u.v.

Experiment		0	10	20	40	60	80	100	120	140
pH 418										
608	Count	1239	889	583	265					
	% S	72	72	47	21					
608	Count	425	418	315	532	1149	1384	878		
	% S		49	37	24	13	6.0	2.0		
605	Count	277		412	334		187	63	47	
	% S			74	12		1.4	.46	.017	
603	Count	149		159		21		28		
	% S			53		3.5		.094		
Average % S			60	53	19	8.3	3.7	.85	.017	
pH 5.4										
606	Count	453	63	89	64	208	198	448		574
	% S		7.4	2.1	.74	.49	.23	.10		.0047
605	Count	210		69	99		250	179	195	
	% S			1.6	.96		.14	.085	.019	
Average % S			7.4	1.9	.85	.49	.19	.093	.019	.0047
pH 6.5										
605	Count	202		199	216		548	260	207	
	% S			.99	.54		.14	.052	.014	
604	Count	175	222	325	180	140	293	368	503	
	% S		2.5	.93	.51	.40	.17	.052	.014	
603	Count	119		114	58					
	% S			.48	.24					
602	Count	117		160		91		86		
	% S			.68		.39		.037		
Average % S			2.5	.77	.43	.40	.16	.047	.012	

Table 6 (continued).

Experiment		Seconds exposure to u. v. 0	10	20	40	60	80	100	120	140
pH 7.2-8.3										
611	Count	742		661	471	561	321			
	% S			.045	.032	.038	.022			
606	Count	417	114	109	124	174	184	411		411
	% S		.13	.025	.029	.042	.022	.0096		.00096
605	Count	155		19	208		548	503	149	
	% S			.023	.067		.042	.013	.0019	
611	Count	697		352	293	300	134			
	% S			.025	.021	.021	.0096			
606	Count	422	92	79	77	79	79	145	529	103
	% S		.12	.018	.018	.018	.0092	.0034	.0012	.00024
605	Count	133		30	147		226	111	26	
	% S			.045	.055		.020	.0033	.00039	
Average % S			.13	.030	.037	.035	.021	.0076	.0012	.00060

Table 7.

Seconds exposure to u. v.

Experiment		0	10	20	40	60	80	100
413	Count	287	127	442	653	558	357	
	% S		44	15	2.3	.20	.012	
415	Count	554	1211	1614	898	1128	1118	
	% S		46	16	2.4	.18	.0077	
423	Count	249	237	226	309	247	130	
	% S		40	19	2.5	.21	.011	
425	Count	266		177	253			
	% S			15	2.1			
499	Count	390		192	76	38	43	
	% S			20	2.0	.095	.011	
Average % S		100	46	17	2.3	.189	.0127	

Table 8.

Seconds exposure to u. v.

Experiment		0	10	20	40	60	80	100	120	140
1.5 minutes exposure to visible light.										
604	Count	212	187	174	226	116	205	316	375	99
	% S		18	4.1	.53	.27	.097	.037	.0089	.0023
603	Count	103		94	87					
	% S			1.6	.37					
593	Count	650			1200	670	303			
	% S				.55	.10	.046			
Average % S			18	2.9	.48	.19	.072	.037	.0089	.0023
2.5 minutes exposure to visible light.										
606	Count	225	95	103	157	141	269	115	158	19
	% S		42	9.2	1.7	.31	.12	.051	.007	.00085
603	Count	149		36	34					
	% S			31	3.1					
593	Count	678			2700	5000	1000	(Approximate)		
	% S				2.0	.74	.15			
Average % S			42	20	2.3	.53	.14	.051	.007	.00085
4 minutes exposure to visible light.										
604	Count	256	165	105	155	149	178	207	107	15
	% S		64	41	12	2.3	.35	.040	.0042	.00059
603	Count	98		38	113					
	% S			31	10					
593	Count	328		478	424	1000	118			
	% S			29	6.5	1.5	.36			
587	Count	490		920	960	418		400	110	
	% S			39	8.2	.71	.07	.07	.02	
586	Count	366		800	800	1100	1900	1500	1300	296
	% S			44	9.0	1.2	.10	.016	.0036	.00081
Average % S			64	37	9.1	1.4	.27	.042	.0093	.00070

Table 9.

Seconds exposure to u. v.

Experiment		0	20	40	60	80	100	120	140
Survival curve for strain B/r.									
385	Count	227	190	160	111	560	224		
	% S		84	70	49	25	9.7		
292	Count	493	372	266	168				
	% S		75	54	35				
511	Count	1057	771	433	207	50			
	% S		73	41	20	4.7			
519	Count	38	29	29	17				
	% S		75	75	45				
519	Count	522	400	233	100				
	% S		77	45	19				
507	Count	1042	643	310	76				
			64	31	7.6				
Average % S			75	51	29	15	7.9	1.9	
Survival curve for strain B, incubated at 37-40° C.									
376	Count	550	268	797	307	784	126	188	34
	% S		49	15	5.5	1.4	.23	.034	.0062
377	Count	554	573	555	540	440	576	703	511
	% S		38	15	5.4	1.1	.24	.043	.014
389	Count	453	354	341		674	693	313	48
	% S		31	11		.89	.15	.01	.0016
390	Count	424			273	488	353		
	% S				6.5	1.2	.083		
526	Count	172	57	59	49	80	64	174	880
	% S		19	6.6	1.4	.45	.071	.019	.0098
557	Count	132	322		339		304	124	
	% S		61		2.6		.092	.0075	
Average % S			40	12	4.3	1.1	.14	.023	.0079

APPENDIX II

Theoretical survival curves.

Two kinds of theoretical curves, the exponential or single-hit curve and the sigmoid or multiple-hit curve, have been used in this discussion. Examples of each type of curve are shown in Figure 10. The exponential curve is obtained in cases where each unit of ultraviolet exposure kills the same proportion of the viable portion of the population. Thus, if a given dose kills 50 percent of the initial population, leaving 50 percent unaffected, a second such dose will kill 50 percent of the survivors, leaving 25 percent surviving. In this way the number of survivors falls off in geometrical progression and the logarithm of the fraction surviving at a given dose is proportional to that dose. Thus,

$$\log_e S = \frac{1}{ad}$$

or,

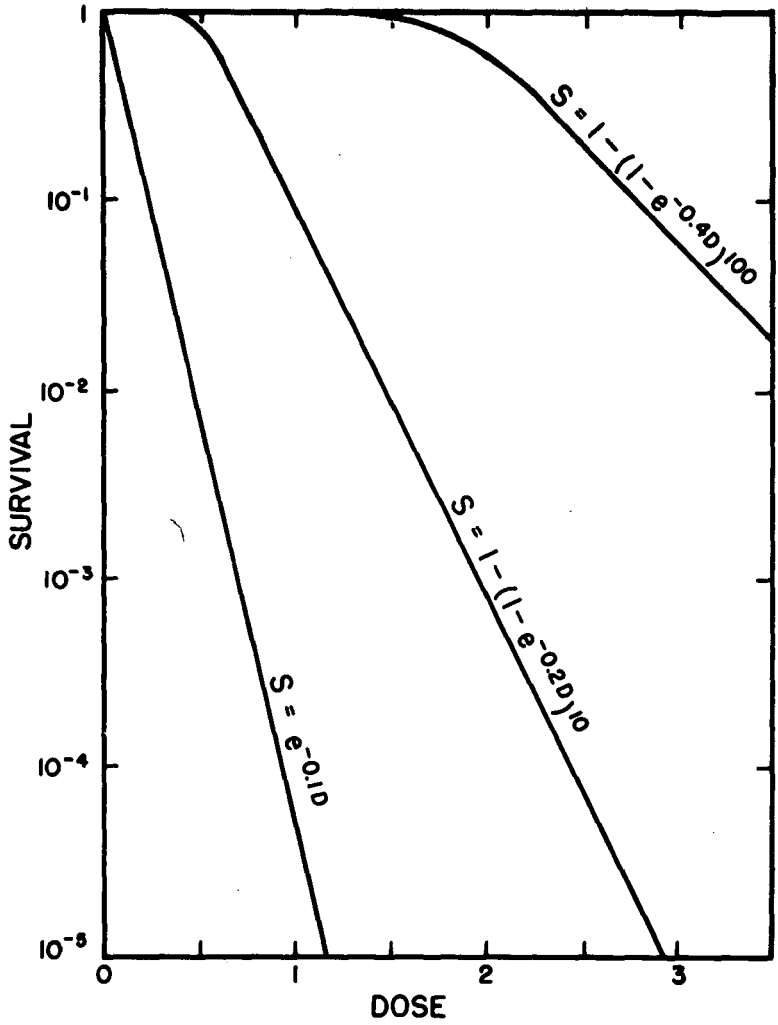
$$S = e^{-ad}$$

where S is the fraction surviving, d is the dose and a is a proportionality constant.

When the accumulation of two or more hits is required to produce an effect, a sigmoid survival curve is obtained. Lea (1946) derives the equation for this sort of curve in the following manner. When the surviving fraction is given by e^{-ad}, then the fraction killed at dose d is $1 - e^{-ad}$. However, if n hits are required to produce the effect, the probability of such an occurrence is $(1 - e^{-ad})^n$, and the surviving fraction is given by the expression

$$S = 1 - (1 - e^{-ad})^n.$$

In most cases it is convenient to plot the experimental curves on semilogarithmic paper. The constants for the equations can be taken directly from the curves by graphical means. The value of a is obtained directly from the slope of the exponential curve or from the straight-line portion of the sigmoid curve. The value of n for a sigmoid curve is found by extrapolating the straight-line portion of the curve to zero dose where n can be read directly from the semilogarithmic scale, (Atwood and Norman 1949).


1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
SURVIVAL
0
1
2
3
DOSE
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$S = 1-(1-e^{-0.2D})^{10}$
$S = e^{-0.1D}$

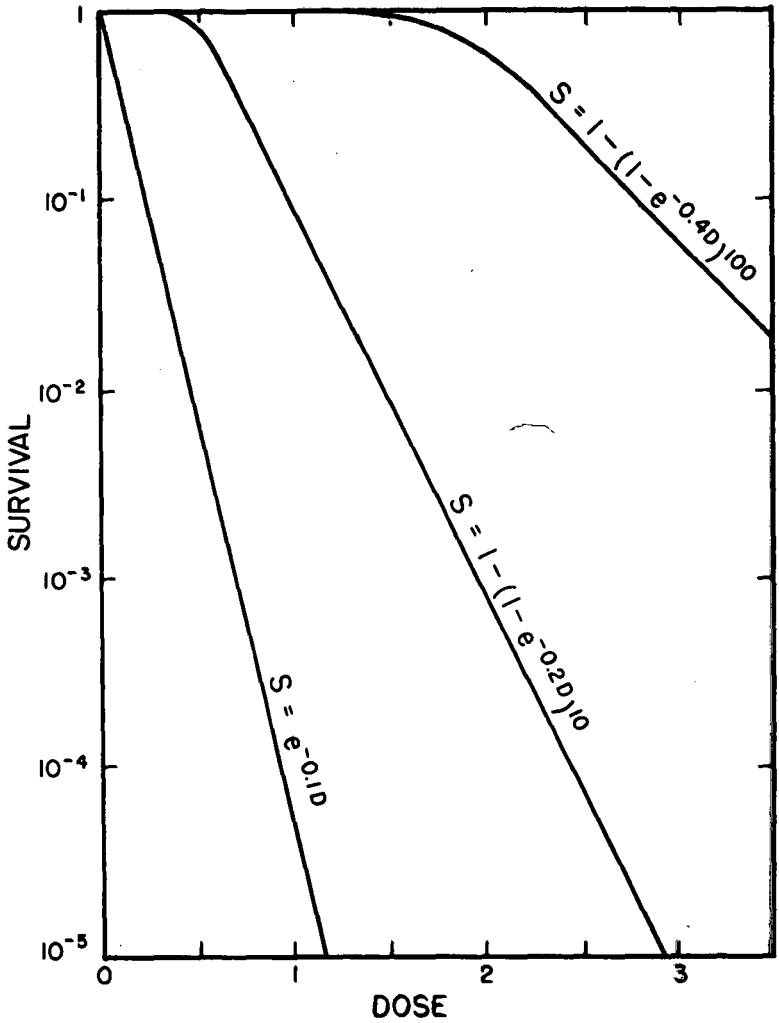

Figure 10. Theoretical single-hit and multiple-hit survival curves.

REFERENCES

Anderson, E.H. 1946 Growth requirements of virus resistant mutants of E. coli strain B. Proc. Nat. Acad. Sci., U. S. 32, 120-128.

Anderson, E. H. 1951 Heat reactivation of ultraviolet-inactivated bacteria. Jour. Bact. 61, 389-394

Atwood, K.C. and A. Norman 1949 On the interpretation of multi-hit survival curves. Proc. Nat. Acad. Sci., U.S. 35, 696-709

Blum, H.F., J.C. Robinson, and G.M. Loos 1950 The loci of action of ultra-violet and X-radiation, and of photorecovery, in the egg and sperm of the sea urchin. Proc. Nat. Acad. Sci., U.S. 36, 623-627.

Dulbecco, R. 1950 Experiments on photoreactivation of u.v. -inactivated bacteriophage. Jour. Bact. 59, 329-347.

Hollaender, A., G. Stapleton, and F.L. Martin 1951 X-ray sensitivity of E. coli as modified by oxygen tension. Nature 167, 103-107.

Kelner, A. 1949 Effect of visible light on the recovery of Streptomyces griseus conidia from ultraviolet injury. Proc. Nat. Acad. Sci., U.S. 35, 73-79.

Kelner, A. 1949 Photoreactivation of ultraviolet-irradiated Escherichia coli with special reference to the dose reduction principle and to ultraviolet-induced mutation. Jour. Bact. 48, 511-522.

Kimball, R.F. and Nenita Gaither 1951 The influence of light upon the action of ultraviolet on Paramecium aurelia. Jour. Cell. and Comp. Physiol. 37, 211-233.

Latarjet, R. and B. Ephrussi 1949 Courbes de survie de levures haploides et diploides soumises aux rayons X. C.R. Acad. Sci., Paris 229, 306-308.

Lederberg, J. 1949 Aberrant heterozygotes in Escherichia coli. Proc. Nat. Acad. Sci., U.S. 35, 178-184.

Lea, D.E. 1946 Actions of radiations on living cells. Cambridge Univ. Press, London, 402 pp.

Luckiesh, M. and T. Knowles 1948 Resistivity of Escherichia coli to ultraviolet energy (2537) as affected by irradiation of preceding cultures. Jour. Bact. 55, 369-372.

Luria, S.E. and M. Delbrück 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28, 491-511.

Novick, A. and L. Szilard 1949 Experiments on light reactivation of ultraviolet inactivated bacteria. Proc. Nat. Acad. Sci., U.S. 35, 591-560.

Roberts, R.B. and Elaine Aldous 1949 Recovery from ultraviolet irradiation in Escherichia coli. Jour. Bact. 59, 363-375.

Rentschler, H.C., R. Nagy, and G. Mouromseff 1941 Bactericidal effect of ultraviolet radiation. Jour. Bact. 41, 745-774.

Witkin, E.M. 1946 Inherited differences in sensitivity to radiation in Escherichia coli. Proc. Nat. Acad. Sci., U.S. 32, 59-68.

Witkin, E.M. 1947 Genetics of resistance to radiation in E. coli. Genetics 32, 221-248.

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