66-14,246

SAVAGE, Norman Lee, 1936-
PHOTOPROTECTION AGAINST X-RAY DAMAGE IN STAPHYLOCOCCUS AUREUS.

The University of Oklahoma, Ph.D., 1966
Bacteriology

University Microfilms, Inc., Ann Arbor, Michigan

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

PHOTOPROTECTION AGAINST X-RAY DAMAGE IN STAPHYLOCOCCUS AUREUS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

BY

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Norman, Oklahoma

1966

PHOTOPROTECTION AGAINST X-RAY DAMAGE

IN STAPHYLOCOCCUS AUREUS

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ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. J. B. Clark for directing the research and aiding in the preparation of the manuscript. Appreciation is also expressed to the other members of the committee for their critical examination and suggestions for the manuscript.

The research was initiated under the sponsorship of a grant from the U. S. Atomic Energy Commission and completed under the sponsorship of a U. S. Public Health Trainee fellowship.

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PHOTOPROTECTION AGAINST X-RAY DAMAGE IN *STAPHYLOCOCCUS AUREUS*

CHAPTER I

INTRODUCTION

One of the most promising approaches for the determination of the mechanisms involved in lethal irradiations of microorganisms has been the use of various methods to alter the end results of these irradiations (Hollaender, 1960). Photoreactivation (Kelner, 1949a; 1949b; Dulbecco, 1950; Jagger, 1958) has been extremely valuable in this respect in the elucidation of ultraviolet action on biological systems.

A related process which may also furnish valuable information on radiobiological mechanisms has been reported in association with protection against ultraviolet damage (Weatherwax, 1956) using *Escherichia coli* strain B. This protection by visible light applied before ultraviolet light was termed photoprotection. Other reports were made previous to this but were subject to some questions. For example, Giese, et al., 1954, reported photoprotection

against ultraviolet damage in Colpidium colpoda when daylight was used as the protecting light, but earlier they had found no effect using blue light (Giese, et al., 1952). An even earlier photoprotective effect was that reported by Cantelmo (1951) on the induction of prophage development in Staphylococcus aureus. The interpretation of these results as photoprotection was questioned by Dulbecco and Weigle (1952) and by Jagger (1958). According to these authors, preillumination of a lysogenic bacterium reduces its ability to support phage growth, and this would result in a lowered amount of observable induction after exposure to ultraviolet light. Photoprotection against ultraviolet damage has now been shown to be a real effect and is discussed by Jagger (1963) in relation to photoreactivation, biological range, chromophore and importance in contributing to a better understanding of the actions of both near and far ultraviolet radiations on the cell.

Photoreactivation against x-ray damage has been attempted, but the results have been negative or have shown only slight reactivation. Dulbecco (1950) reported slight photoreactivation of phage T2 inactivated by x-rays in nutrient broth. Watson (1950; 1952) reported similar results with T2, T4, and T6. Positive results on the photoreactivation of induction of prophage development in Bacillus megaterium after x-rays (Latarjet, 1951) were later found

due to an arrest of virus development by visible light applied after x-rays and were not a true reversal of the induction process (Latarjet, 1954). Other attempts to show photoreactivation of x-ray damage have been negative for induction of prophage development in Pseudomonas pyocyanea (Tobin, 1953), reduced vigor after autogamy in Paramecium aurelia (Kimball and Gaither, 1950), and cleavage delay in the fertilized egg or inactivation of the sperm of Arbacia punctulata (Blum, et al., 1951).

In view of this failure to demonstrate the existence of photoreactivation of x-ray damage, it is somewhat surprising to find positive results with photoprotection against x-ray damage. Clark and Frady (1961) reported a photoprotective effect against the lethal action of x-ray in Nocardia corallina. Although the preillumination of the organism with visible light resulted in some killing itself, the possibility of selection of resistant organisms was discounted on the basis of decay of the photoprotection after storage of the illuminated cells in saline for five hours or less. These cells, which no longer showed the photoprotective effect after storage, could be photoprotected again by a second visible light exposure identical to the first. The process was also reported to be x-ray dose rate dependant.

Following this first report, additional investigations

indicated that photoprotection could be demonstrated in several microorganisms (Raizen, 1963) of different taxonomic positions. Initial experiments on the visible light action spectrum indicated that wavelengths of 450 mμ and less were responsible for the effect. This spectrum was similar to that reported by Jagger and Stafford (1962) for photoprotection against ultraviolet damage. Klein and Klein (1965) have now confirmed photoprotection against genetic damage caused by x-ray in Neurospora crassa conidia. No protection against the lethal effect of x-rays was found, nor was there any x-ray dose rate dependency.

Investigations on photoprotection against x-ray damage in Staphylococcus aureus indicated that there were a number of variables which affect the amount of protection obtained as well as whether any protection can be obtained (Savage, et al., 1964). The physiological state of the organism as determined by cell age, culture medium, and incubation temperature all seem to alter the reaction of the cell with respect to its photoprotective capacity as well as x-ray sensitivity. It is of interest to note that this organism is also sensitive to the visible illumination used in photoprotection studies.

The sensitivity of biological systems to irradiation with visible light has been observed rather irregularly for a number of years (Hollaender, 1943). Many of these observations are subject to criticism on the basis of the

experimental technique since several of the results can now be explained as due to the action of far ultraviolet rather than near ultraviolet or visible irradiations. Hollaender (1943) compared the effects of wavelengths from 350 to 490 mμ with the effects of wavelengths from 218 mμ to 295 mμ on Escherichia coli. He observed a killing effect with the longer wavelengths which presented a threshold type killing curve, a temperature coefficient of 1.7-2.2, retarded growth phase, toxicity to storage in salt solutions, and no mutagenic effect. Similar results have been reported for other bacteria and yeast (Hollaender, et al., 1938), fungi (Hollaender and Emmons, 1941), and nematode eggs (Jones and Hollaender, 1942).

This investigation was initiated, essentially, to determine more of the characteristics of the photoprotective effect against x-ray damage. The effects of various environmental conditions on the process were determined. In addition, in view of the reoccurring existence of the light sensitivity of many of the organisms which are photoprotectable, further studies on the possibility of a selective rather than a protective effect were done. And, lastly, a correlation was made between the effect of visible light on the growth of the organism and its possible relation to photoprotection.

CHAPTER II

MATERIALS AND METHODS

Culture. Staphylococcus aureus, obtained from the University of Oklahoma stock culture collection, was used in this study. The stock culture was maintained on either nutrient agar or brain heart infusion agar. Prior to an experiment the organism was transferred at 24 hour intervals for several successive transfers to assure a stable, active culture. Normally, the incubation temperature was 37 C unless otherwise indicated.

X-irradiation. The source of x-rays was a Machlett OEG-60 x-ray tube with a beryllium window operating at 70 kv and a ma setting to give the desired dose rate. A dose rate of 300 r/min was used since this has been shown to be optimum for photoprotection (Clark and Frady, 1961). Dose rate measurements were made with a Victoreen Model R rate meter.

Visible light source. A Bausch and Lomb electronic-feed carbon arc lamp equipped with a water cell and a heat absorbing filter was used as the source of protecting light. Normally, the lamp was adjusted to give a dose rate of 400 ergs/sec/mm^2 which is optimum for photoprotection at

this dose rate (Howell, unpublished data). Energy measurements were made with a calibrated Eppley thermopile. This is a circular thermopile with eight junctions, bismuth silver elements, lampblack coating, a 7/16 inch diameter shutter opening and a calcium fluoride window. The output from the thermopile was measured with a Keithley Model 150A microvoltmeter. Variations in the light intensity during exposure were recorded and then averaged to give the energy measurements.

Experimental procedure. The organism was grown on agar slants for several successive 24 hour transfers. A 24 hour culture was washed from the slant with 0.9 % saline and inoculated into broth of the same type as the agar. The size of the inoculum was standardized to an optical density of 0.2 on a Bausch and Lomb Spectronic 20 spectrophotometer. The culture was then incubated and samples removed at appropriate time intervals for experimentation. The samples were diluted in saline to give approximately 2×10^4 cells/ml. This was then divided into 5 ml samples and placed in 50 ml Kimax crystallizing dishes. Clumps were eliminated in the sample by shaking with glass beads on a Vortex Jr. mixer. The samples were stirred magnetically during the irradiations to assure an even exposure. Temperatures of approximately 6 C were maintained by an ice bath and monitored with a thermister probe. The number of survivors was determined from plate counts, with triplicate samples for each condition and 3-5 plates per sample.

Anaerobic growth and irradiation under anoxic conditions. Growth of slants in a nitrogen atmosphere was achieved with Brewer anaerobic jars which were evacuated 3-5 times and flushed each time with nitrogen. Oxygen contaminating the tank nitrogen was removed by passage through an alkaline pyrogallol solution (Skerman, 1959). Broth cultures were bubbled with oxygen-free nitrogen during growth. Prickett tubes, capped with paraffin, were used for anaerobic growth after x-irradiation.

Irradiation under anoxic conditions was done by bubbling nitrogen through the samples which were covered with Saran wrap. A small hole in the cover allowed insertion of a small glass tube for the addition of the nitrogen. A period of 5-10 minutes equilibration with nitrogen was permitted before the actual irradiations. The cells irradiated anoxically were suspended in 0.2 M phosphate buffered saline (pH 6.8).

Successive and constant irradiations. Growth under constant illumination was accomplished by placing the organisms on agar and in broth in a water bath at 37 C, illuminated by a 100 watt incandescent light bulb from a distance of 10-15 cm. Organisms treated in this manner were then subjected to the previously described irradiation procedure. The survivors were incubated in the dark.

Successive visible and x-irradiations were done by pooling survivors of these irradiations from agar after growth for 15-20 hours and reinoculating a fresh agar slant. These

were allowed to incubate for 24 hours, harvested and inoculated into broth, and appropriate samples removed for subsequent irradiations.

Growth studies. Growth of the organism after visible and x-irradiation, singly and in combination, was followed turbidimetrically and by plate count. For the turbidity changes, the irradiated organisms were placed in standardized cuvettes containing brain heart infusion broth and changes in optical density measured with a Bausch and Lomb Spectronic 20 spectrophotometer. The only alteration in general procedure for these cells was that they were washed once in 0.9 % saline and stored at room temperature for approximately 30 minutes prior to the irradiations, a procedure found to decrease the lethal effect of visible light.

CHAPTER III

RESULTS

Effect of Environmental Factors on Photoprotection

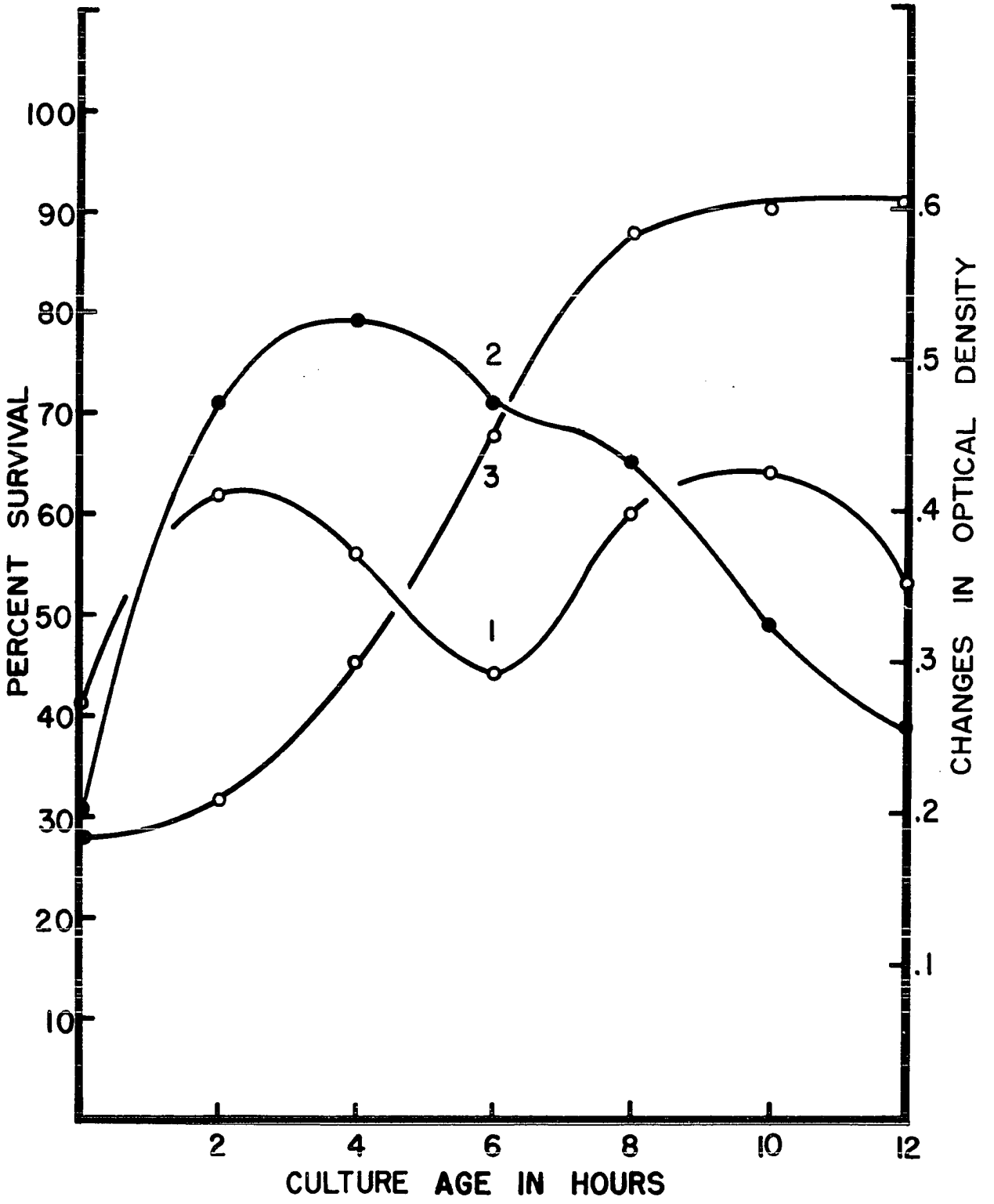
One of the difficulties encountered in the study of photoprotection against x-ray damage has been the inability to acquire reproducible results. This, coupled with the relatively small amount of protection afforded to a number of the test organisms (Raizen, 1963), stimulated an attempt to determine a better characterization of the phenomenon in relation to reproducibility as well as to increase the amount of protection. Early experiments on this phenomenon were conducted using stationary phase agar slant cultures (Clark and Frady, 1961; Collard, 1961; Raizen, 1963). It seemed feasible that the age of the organism, as well as the culture medium and other environmental factors, might be of importance in determining the end result of photoprotection. Therefore, a study of environmental factors on the phenomenon was initiated.

Figures 1 and 2 show the results obtained on cells of different ages grown in nutrient broth and on nutrient agar (Fig. 1) and brain heart infusion broth and agar (Fig. 2)

Figure 1. Variation of x-ray sensitivity and photoprotection with culture age.

Curve 1 shows the response of control cells (x-ray only), and curve 2 shows the response of cells exposed to visible light prior to x-ray. The cells were grown on nutrient agar and broth at 37 C. Curve 3 is a typical growth curve under these conditions.

For Figures 1-9 the x-ray dose rate was 300 r/min for a total dose of 4500 r. The visible light dose rate was 400 ergs/sec/mm^2 for a total dose of 3.5 X 10^5 ergs/mm^2.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CULTURE AGE IN HOURS
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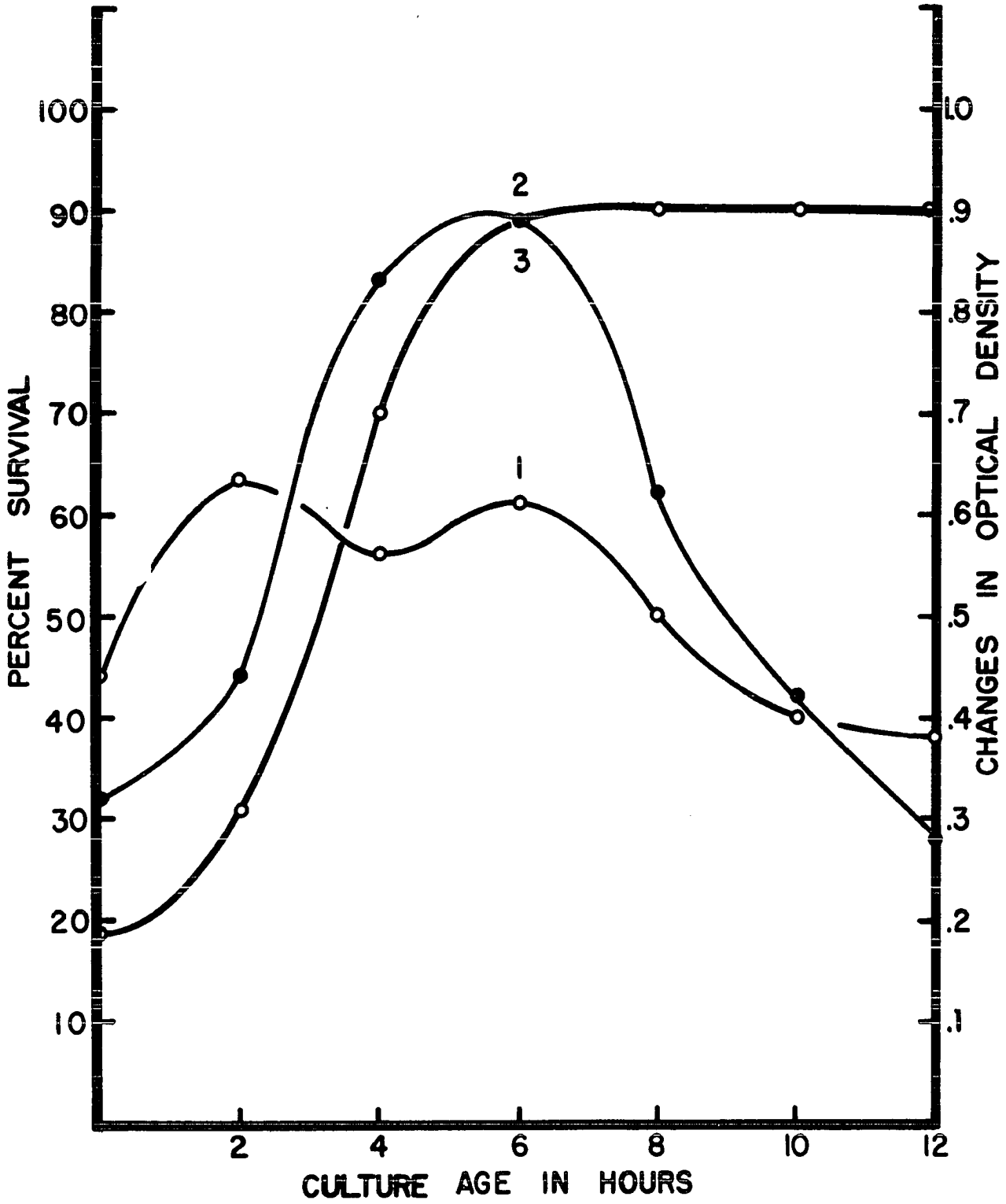
Figure 2. Variation of x-ray sensitivity and photoprotection with culture age.

Curve 1 shows the response of control cells (x-ray only), and curve 2 shows the response of cells exposed to visible light prior to x-ray. The cells were grown on brain heart infusion agar and broth at 37 C. Curve 3 represents a typical growth curve under these conditions.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which, in comparison to nutrient broth and agar, stimulates the growth rate and total growth of the culture.

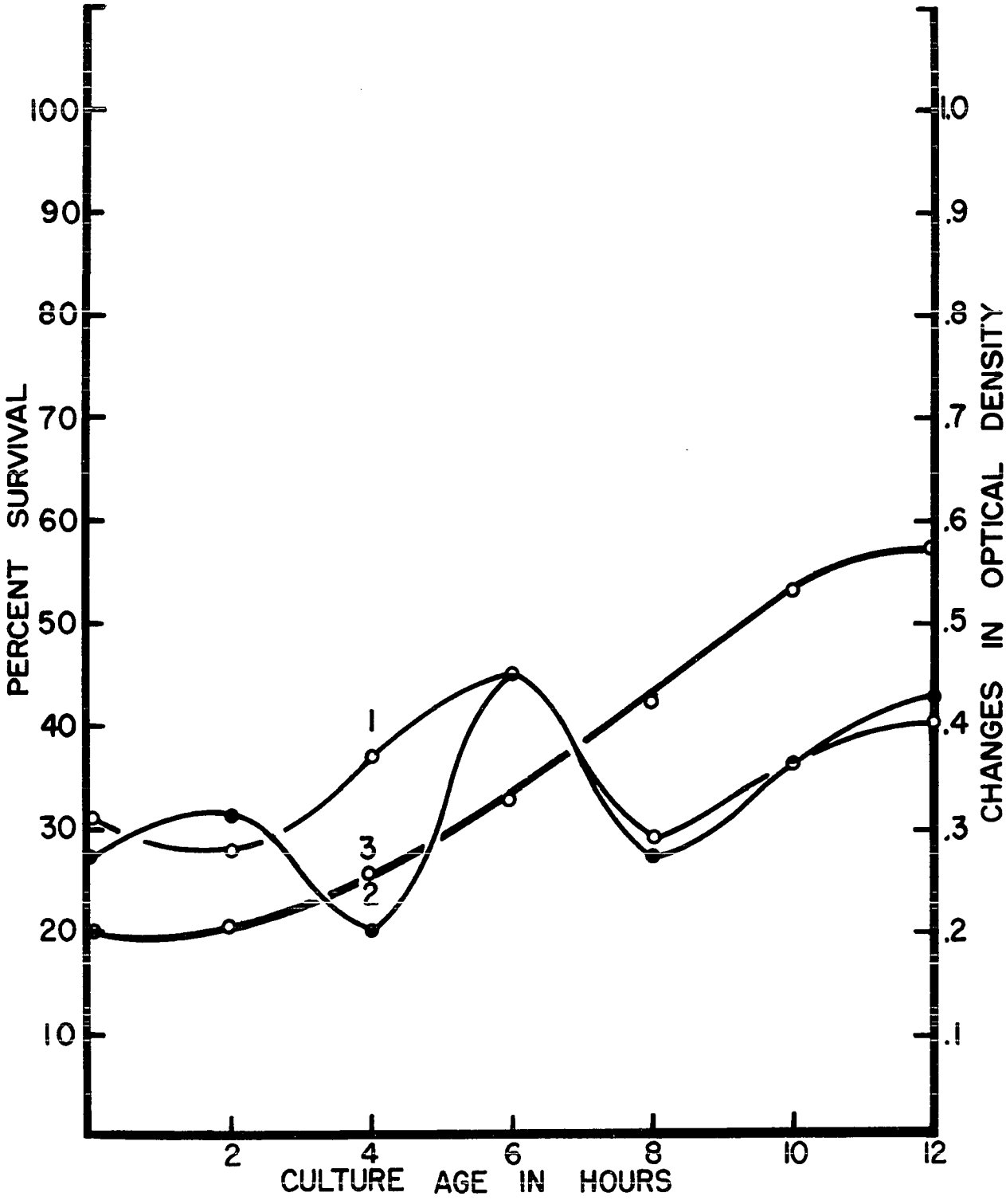
Consideration of Figure 1 indicates that one reason for the variability of previous results was the age of the culture. These results show that the maximum amount of protection is afforded the cells by visible light between 4 and 6 hours of growth after inoculation into fresh medium. This protection is preceded and followed by an apparent sensitization to x-ray by the visible light. The increase in the relative amount of photoprotection during logarithmic growth is due to a larger survival of the illuminated sample as well as an increase in the sensitivity of the non-protected cells to x-irradiation. A similar variation in sensitivity to x-irradiation during the growth cycle has been reported previously by Stapleton (1955a; 1955b) in Escherichia coli.

A comparison of Figures 1 and 2 indicates a similar response when the organism is grown on brain heart infusion. An increase in survival of the illuminated sample indicating more protection is practically nullified by the increase in x-ray resistance of the nonprotected cells. The result is that approximately the same amount of protection as in Figure 1 is obtained. The two-peaked control survival curve is shifted slightly to the left due to the increased growth rate of the organisms in this medium.

Figure 3 shows the results obtained when the organism was incubated at 29 C as compared to 37 C shown in Figure 1.

Figure 3. The effect of incubation at 29 C on photoprotection and x-ray sensitivity of cells of different ages.

Curve 1 shows the response of control cells (x-ray only), and curve 2 shows the response of cells exposed to visible light prior to x-ray. The cells were grown on nutrient agar and broth. Curve 3 shows a typical growth curve under these conditions.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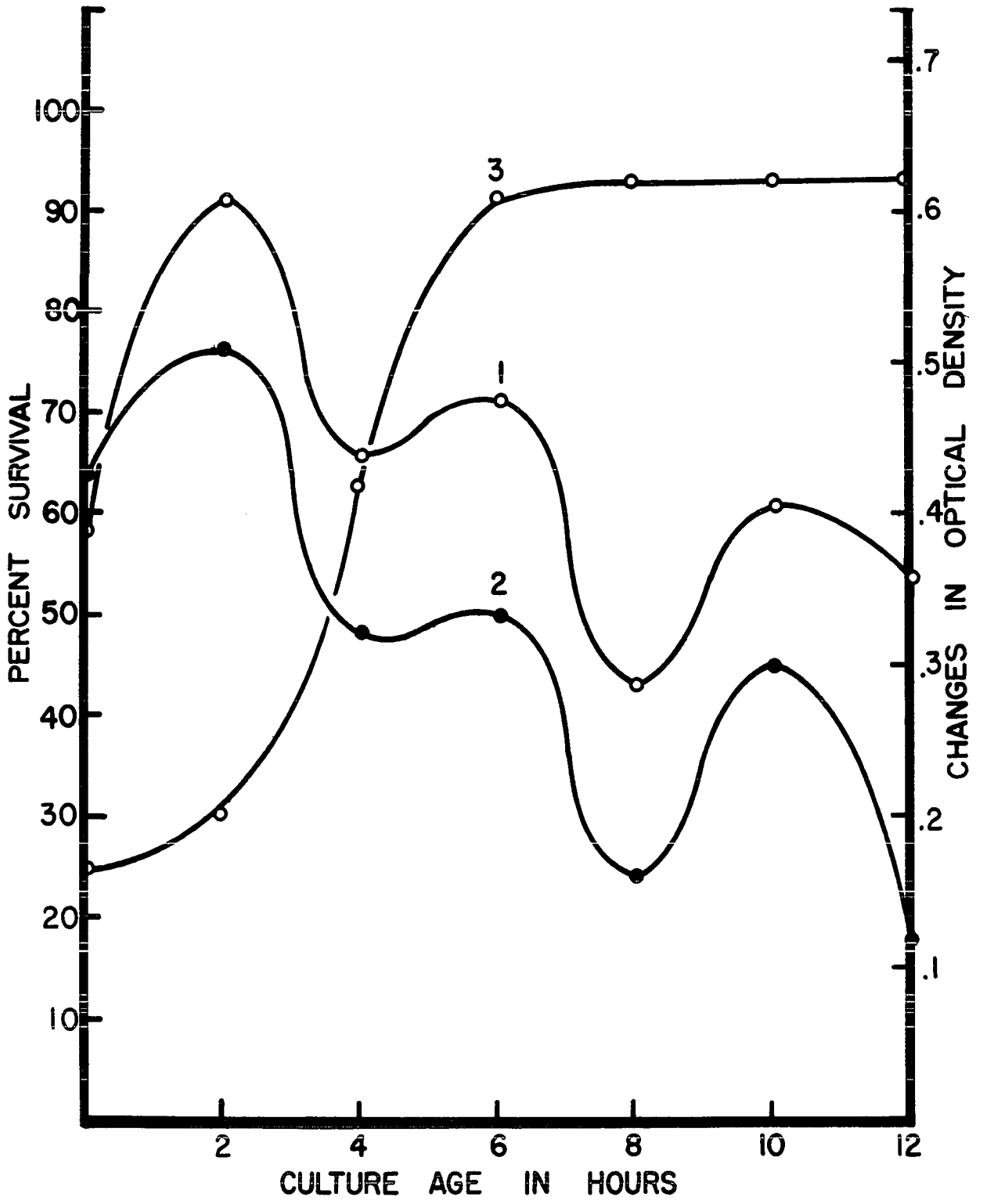
No photoprotection was obtained during the 12 hour growth period studied. This was consistent with experiments on Serratia marcescens in which a small amount of protection was found if the organism was cultured at 37 C but none at 29 C. Raizen (1963) reported Serratia marcescens as one of the organisms which could not be photoprotected; however, the age of the culture may again have been the determining factor. It should be noted that the survival curve of S. aureus incubated at 29 C is shifted to the right due to the slower growth rate at this temperature, so that the phase of growth is also shifted in this direction. There is, also, an increase in the overall x-ray sensitivity which may contribute to the lack of any photoprotection.

Effect of Anaerobic Growth and Irradiation under Anoxic Conditions on Photoprotection

Another factor which has been studied extensively in relation to modification of x-ray sensitivity is oxygen tension. Studies in bacterial systems have shown that a reduced oxygen tension results in an increased radioresistance (Hollaender et al., 1951). The effect of anaerobic culturing was also found to be increased resistance. Therefore, experiments were designed to determine the effect of anaerobic culturing and oxygen tension during irradiations on photoprotection. The results from these experiments are shown in Figures 4-8. Figure 4 shows the results obtained with the organism cultured anaerobically, under nitrogen, during

Figure 4. The effect of anaerobic growth and irradiations under anoxic conditions on photoprotection and x-ray sensitivity of cells of different ages.

Curve 1 shows the response of control cells (x-ray only), and curve 2 shows the response of cells exposed to visible light prior to x-ray. The cells were grown under a nitrogen atmosphere before and after the irradiations on brain heart infusion and irradiated under nitrogen. The incubation temperature was 37 C. Curve 3 shows a typical growth curve under these conditions.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the entire experiment and irradiated under a nitrogen atmosphere. The incubation temperature was 37 C. Photosensitization was the result with all samples. Instead of a two-peaked x-ray survival curve for the control cells described previously, a three-peaked curve is seen. In addition, the overall sensitivity of the cells to the x-ray exposure decreased with the exception of the 8 hour sample.

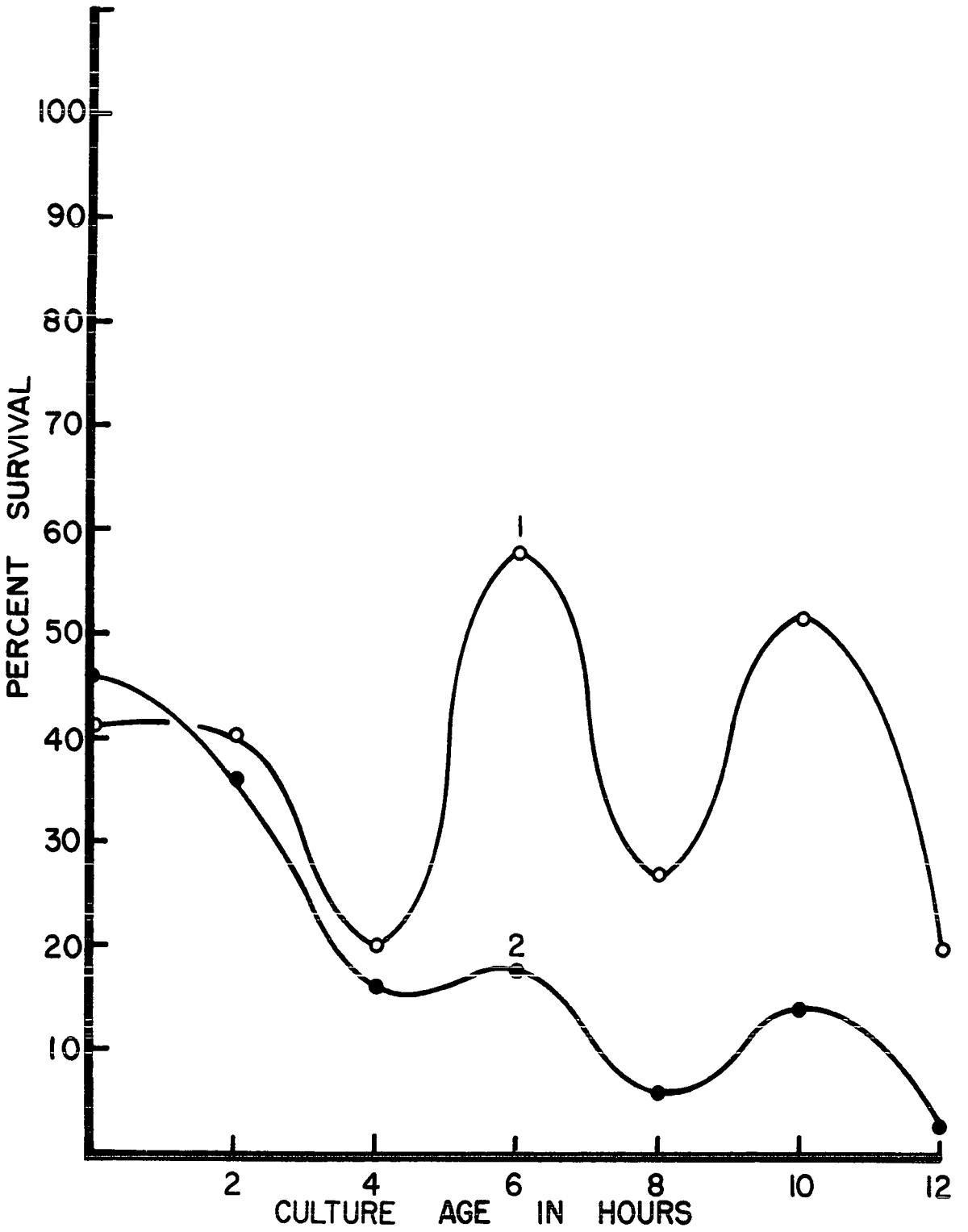
In view of this loss of photoprotection under these conditions, it became important to determine what specific condition was responsible for this loss. Figure 5 shows the results obtained when the cells were treated as described for the preceding experiment with the exception that the cells were incubated aerobically after irradiation. Again no photoprotection was obtained. Approximately the same type of survival curve was obtained as with the preceding experiment, but the sensitivity of the cells to x-ray was increased.

Anaerobically grown organisms irradiated under air can be photoprotected (Fig. 6). In addition, the survival curve of the control cells has the same shape as the system cultured and irradiated under air (Fig. 1). The main differences noted under these conditions are a reduced amount of photoprotection and an overall increase in x-ray sensitivity.

Since photoprotection was obtained with an anaerobic culture when irradiated under air, the critical point for

Figure 5. The effect of aerobic recovery of anaerobically grown and anoxically irradiated cells on photoprotection and x-ray sensitivity of cells of different ages.

Curve 1 shows the response of control cells (x-ray only), and curve 2 shows the response of cells exposed to visible light prior to x-ray. The cells were grown under a nitrogen atmosphere prior to the irradiations but aerobically after. The cells were under a nitrogen atmosphere when irradiated. The growth medium was brain heart infusion, and the incubation temperature was 37 C.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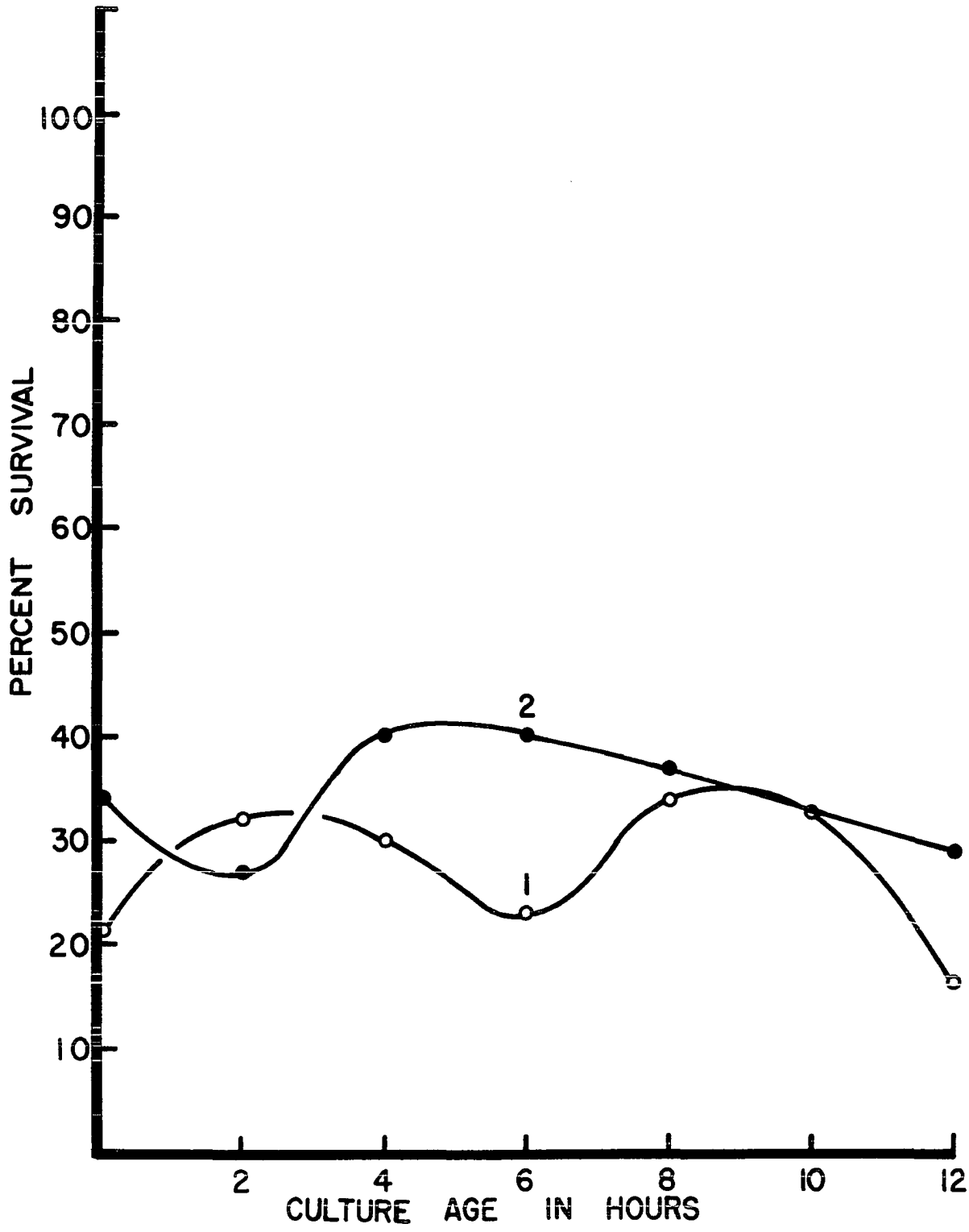
CULTURE AGE IN HOURS

Figure 6. The effect of aerobic irradiations and recovery of anaerobically grown cells on x-ray sensitivity and photoprotection of cells of different ages.

Curve 1 shows the response of control cells (x-ray only), and curve 2 shows the response of cells exposed to visible light prior to x-ray. The cells were grown under nitrogen on brain heart infusion agar and broth prior to the irradiations but aerobically after. The cells were irradiated under air.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CULTURE AGE IN HOURS

protection seemed to be the conditions of the actual irradiation. In order to substantiate this, a culture was grown aerobically before and after the irradiations, but the irradiations were done under a nitrogen atmosphere (Table 1). Since the effects of cell age on photoprotection were determined previously for aerobically grown cells, it did not seem necessary to test cells of various ages. Instead, a culture in log phase (5 hours) which would give maximum photoprotection was used. The amount of photoprotection under normal (air) irradiations for this culture was determined as well as irradiations under nitrogen. It can be seen that visible irradiations under nitrogen followed by x-irradiations under air results in photoprotection; however, x-irradiation under nitrogen preceded by visible irradiation under air results in photosensitization. The amount of protection with the visible irradiation under anoxic conditions is approximately equal to that obtained under normal conditions with this particular culture.

Selection as a Factor in Photoprotection

One observation that was made during the early phase of this investigation on photoprotection was the apparent sensitivity of the organism to visible light. A comparison of the relative amount of photoprotection through the growth cycle (Fig. 7), obtained by subtracting the nonprotected survival curve from the protected survival curve (Fig. 2), with the relative amount of killing by visible light (Fig. 8) indicated a correlation between maximum photoprotection and maximum sensitivity to visible light. This correlation plus

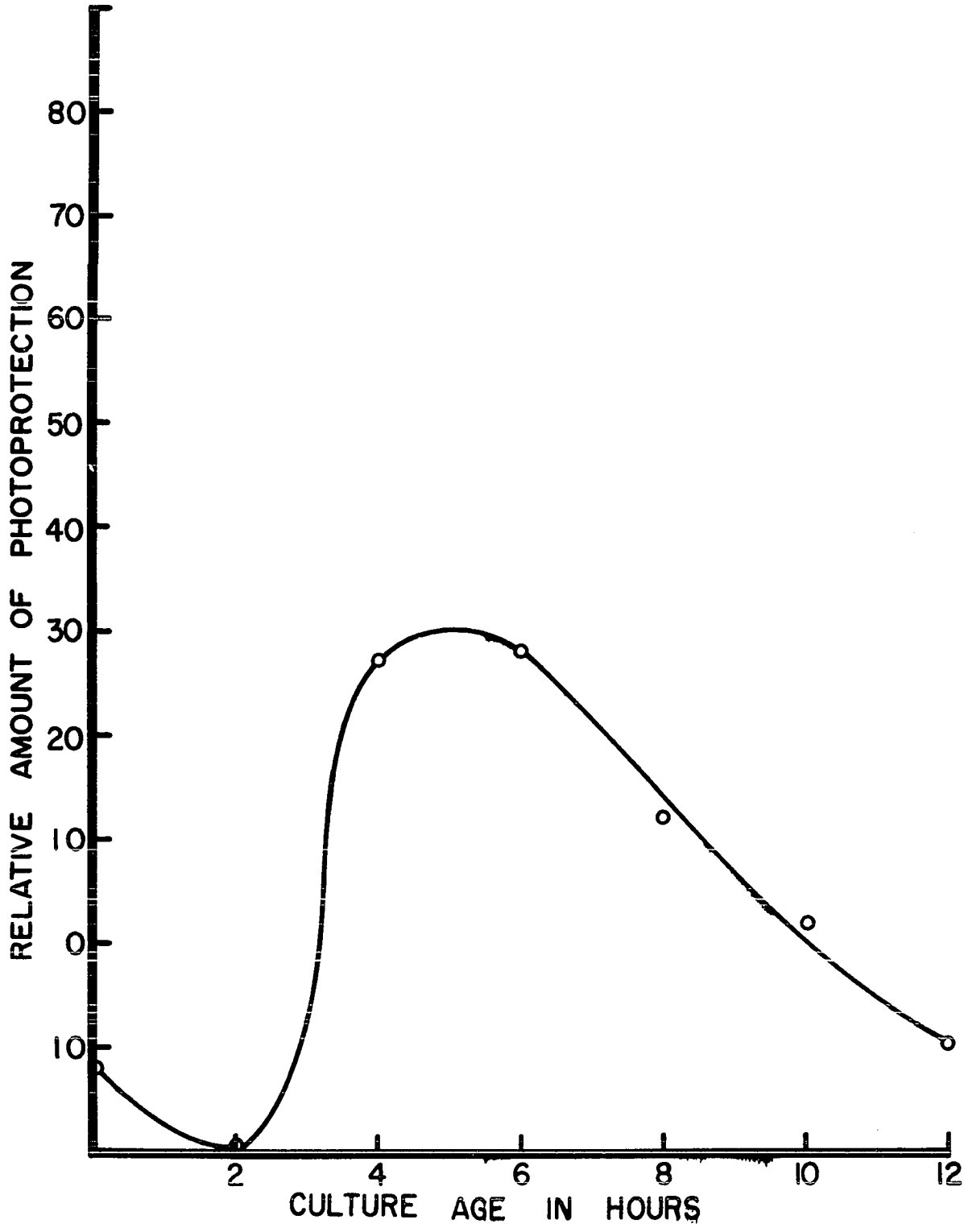
TABLE I

The effect of anoxic conditions during visible or x-irradiation on photoprotection of a 5 hour, aerobic, brain heart infusion broth culture.

Radiation Conditions	Percent Survival
X-ray under air	58
Visible and x-ray under air	73
X-ray under air	64
Visible, N_2; x-ray, air	81
X-ray under N_2	55
Visible, air; x-ray, N_2	40

Figure 7. The effect of culture age on the relative amount of photoprotection.

The curve was obtained by subtracting the percentage survivors of the control samples (curve 1) from the photoprotected samples (curve 2) in Figure 2.


RELATIVE AMOUNT OF PHOTOPROTECTION
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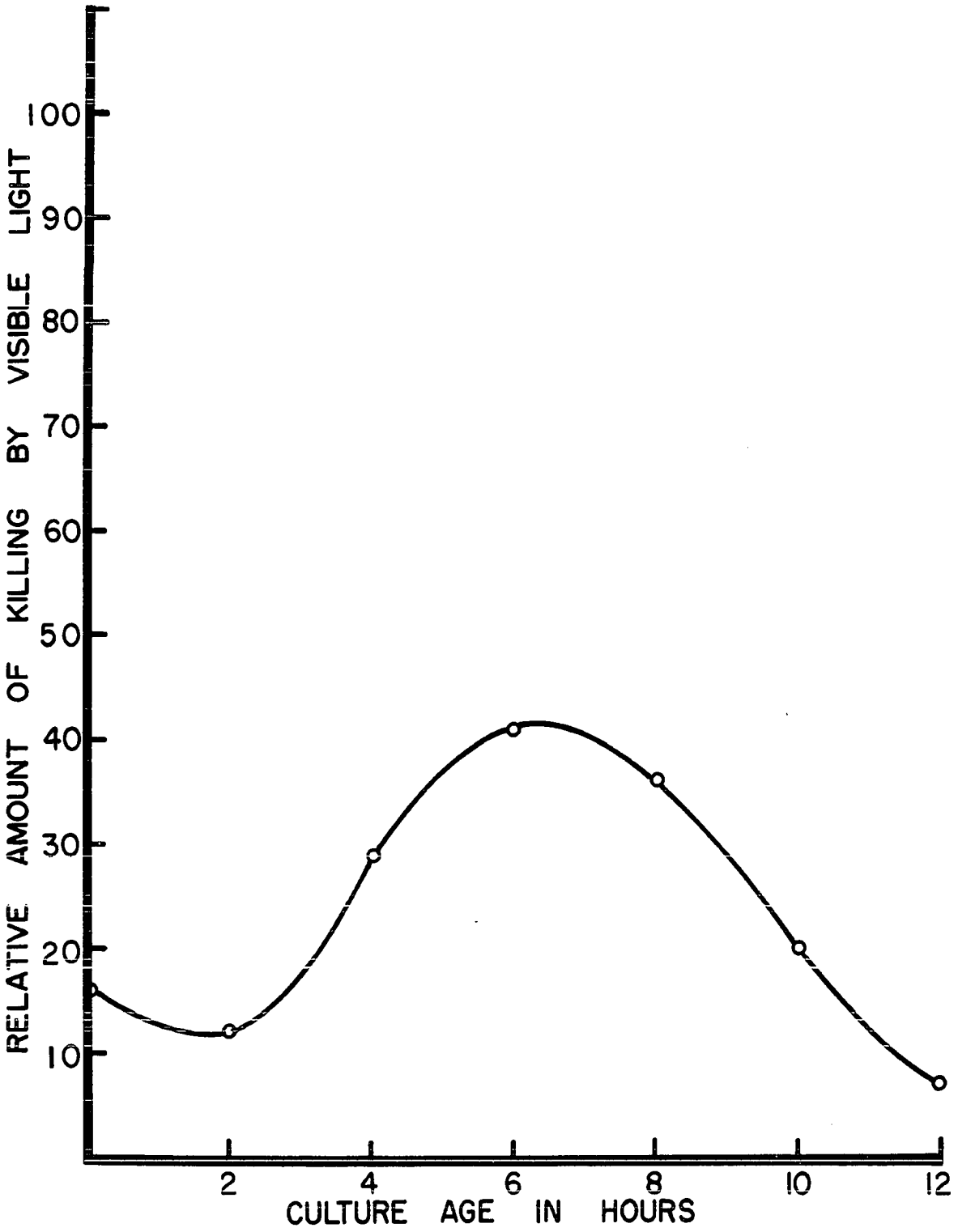
CULTURE AGE IN HOURS

Figure 8. The effect of culture age on the relative amount of killing by visible light.

The curve was obtained by subtracting the percentage survivors of the samples stirred and illuminated from a control sample that was stirred only.


RELATIVE AMOUNT OF KILLING BY VISIBLE LIGHT
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CULTURE AGE IN HOURS

the cyclic changes in x-ray sensitivity and the variability encountered in experimental results were all indications of a mixed culture. The possibility of selection under these conditions was very distinct, therefore, in contrast to previous reports (Clark and Frady, 1961; Collard, 1961).

Three approaches were used in an attempt to determine whether photoprotection is, in fact, selection. First, the cells were grown under constant illumination with the basic assumption that growth of sensitive cells would be inhibited, allowing the development of a visible light resistant population. These organisms were then tested for photoprotection, light sensitivity, and x-ray sensitivity (Figures 9, 10, and 11). It may be noted in Figure 9 that one 24 hour growth period under constant illumination results in a slight increase in the amount of photoprotection obtained, followed by a decrease until, after 3 transfers, no photoprotection is demonstrable. A comparison of these results with those pertaining to visible light sensitivity (Fig. 10-1) eliminates selection as being responsible for photoprotection since the culture is not sensitive to visible light after a single 24 hour growth period, but the cells are still photoprotectable.

The results of continuous visible irradiation on x-ray sensitivity (Fig. 11) are helpful in explaining the previously described effects on photoprotection and visible light sensitivity. Growth of S. aureus under constant

illumination results first in an increased radiosensitivity followed by a rather drastic decrease. This coincides with the changes in photoprotection and light sensitivity, indicating that the first period of growth under constant illumination results in a population resistant to visible light but more photoprotectable than non-illuminated cells. The low-intensity constant illumination would be expected to afford some protection to the cells which might be additive to the short period of maximum photoprotecting light, resulting in an increase in photoprotection. The results for x-ray sensitivity are, however, contrary to what would be expected since the sensitivity of the control cells should decrease with the continuous visible irradiation. This low dose rate of illumination appears, in effect, to sensitize some of the cells to x-ray and still have an additive effect on photoprotection when the cells are exposed to a maximum photoprotecting dose. Continued visible illumination would be expected, eventually, to reach a maximum photoprotecting dose so that further visible irradiations would be without effect. This would result in an x-ray sensitivity decrease for the total population plus a loss of any effect of additional visible light. That this occurs is deducible from the results in Figures 9 and 11.

A second approach to the problem of selection was to culture survivors of visible irradiation with the expectation that a visible light resistant population would

eventually be obtained. A suspension of a 5 hour culture was exposed to a maximum photoprotection dose of visible light (3.5×10^5 ergs/mm^2) which reduced the number of viable cells to about 70 % of the original. These survivors were incubated for 15-20 hours, harvested and pooled. This was then used as an inoculum for fresh agar slants. These agar slant cultures were incubated for 24 hours, harvested and reinoculated into broth. A 5 hour broth culture was then used to repeat this procedure. The cultures resulting from the visible light selections were tested for photoprotection, light sensitivity, and x-ray sensitivity (Figures 9-2, 10-2, and 12). The results from the successive visible light selections are, after a single selection, opposite to those obtained with continuous illumination. The cells are not photoprotectable but their sensitivity to visible light is increased, possibly to such a degree as to mask any photoprotection. In addition, the x-ray sensitivity of the cells selected by successive visible light exposure decreased. This indicates some retention of the protective action of visible light during the growth period between the successive visible light exposures, a process that would not be expected since it was reported that photoprotection decays during 5 hour storage (Clark and Frady, 1961; Collard, 1961). If photoprotection were a selective effect, it would be expected that prior visible light exposure would result in an increase in visible light resistance and a loss of photoprotectability.

However, these results are contradictory to this since the maximum amount of visible light killing did not result in any photoprotection.

Selection of resistant cells by successive x-ray exposures, using the same procedure described for visible light selection, resulted in an increase in photoprotection after one exposure followed by a decrease (Fig. 9-3). After 3 selections a small amount of photoprotection was again demonstrable. Visible light sensitivity was more gradually eliminated than in the two previous methods but was complete after three successive x-irradiations (Fig. 10-3). As in the case of visible light selection, the cells became more resistant to x-ray (Fig. 13).

All of these results on selection indicate that the cell population is not homogeneous with respect to sensitivity to visible light, x-ray or photoprotectability. This is not surprising in view of the fact that studies on photoreactivation of ultraviolet damage are concerned with only a certain sector of the population susceptible to the process (Rupert and Harm, 1966). These studies, although not completely explainable, do indicate that the process of photoprotection against x-ray damage is, in fact, real, and not merely a selection of x-ray resistant cells by visible light.

Effect of Visible and X-irradiations on Growth Rate

Demonstration of photoprotection in S. aureus is best accomplished when the cells are in the late log phase

Figure 9. The effect of continuous illumination, visible light selection, and x-ray selection on the relative amount of photoprotection.

Curve 1 shows the results obtained with cells grown under continuous, low intensity illumination before normal treatment for photoprotection. The abscissa represents the number of previous 24 hour growth periods under continuous illumination.

Curve 2 shows the results obtained with cells surviving successive visible light doses of 3.5×10^5 ergs/mm^2 at 48 hour intervals. The abscissa represents the number of previous visible light exposures.

Curve 3 shows the results obtained with cells surviving successive x-ray doses of 4500 r at 48 hour intervals. The abscissa represents the number of previous x-ray exposures.

The cells used were from a 5 hour brain heart infusion broth culture. The curves were obtained by subtracting the control from the photoprotected samples.

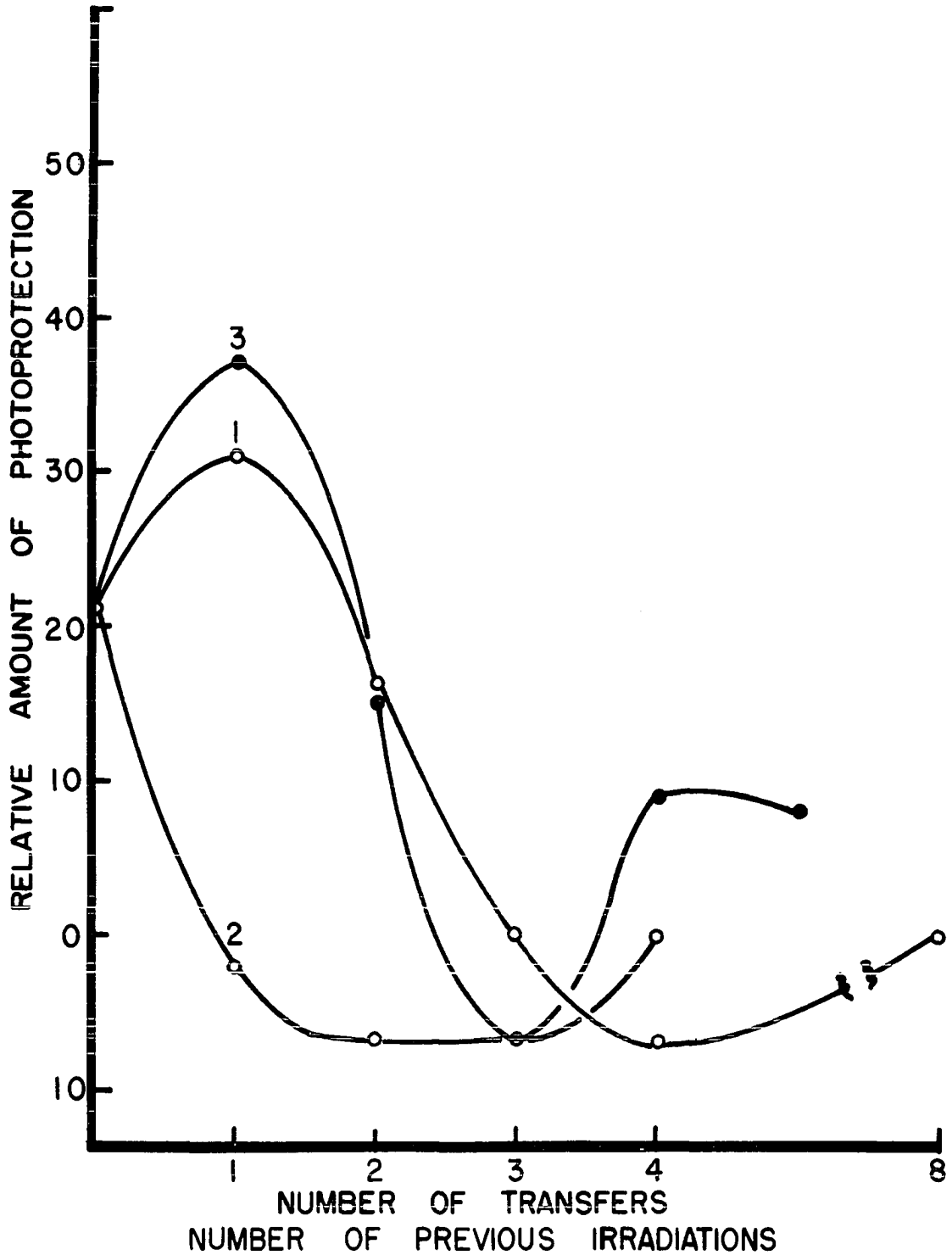

RELATIVE AMOUNT OF PHOTOPROTECTION
50
40
30
20
10
0
10
3
1
2
1
2
3
4
8
NUMBER OF TRANSFERS
NUMBER OF PREVIOUS IRRADIATIONS

Figure 10. The effect of continuous illumination, visible light selection, and x-ray selection on the relative amount of visible light killing.

Curve 1 shows the results obtained with cells grown under continuous, low intensity illumination before exposure to a visible light dose of 3.5×10^5 ergs/mm^2. The abscissa represents the number of previous 24 hour growth periods under continuous illumination.

Curve 2 shows the results obtained with cells surviving successive visible light doses of 3.5×10^5 ergs/mm^2 at 48 hour intervals. The abscissa represents the number of previous visible light exposures.

Curve 3 shows the results obtained with cells surviving successive x-ray doses of 4500 r at 48 hour intervals. The abscissa represents the number of previous x-ray exposures.

The cells used were from a 5 hour brain heart infusion broth culture. The curves were obtained by subtracting the survivors of the illuminated sample from the control sample.

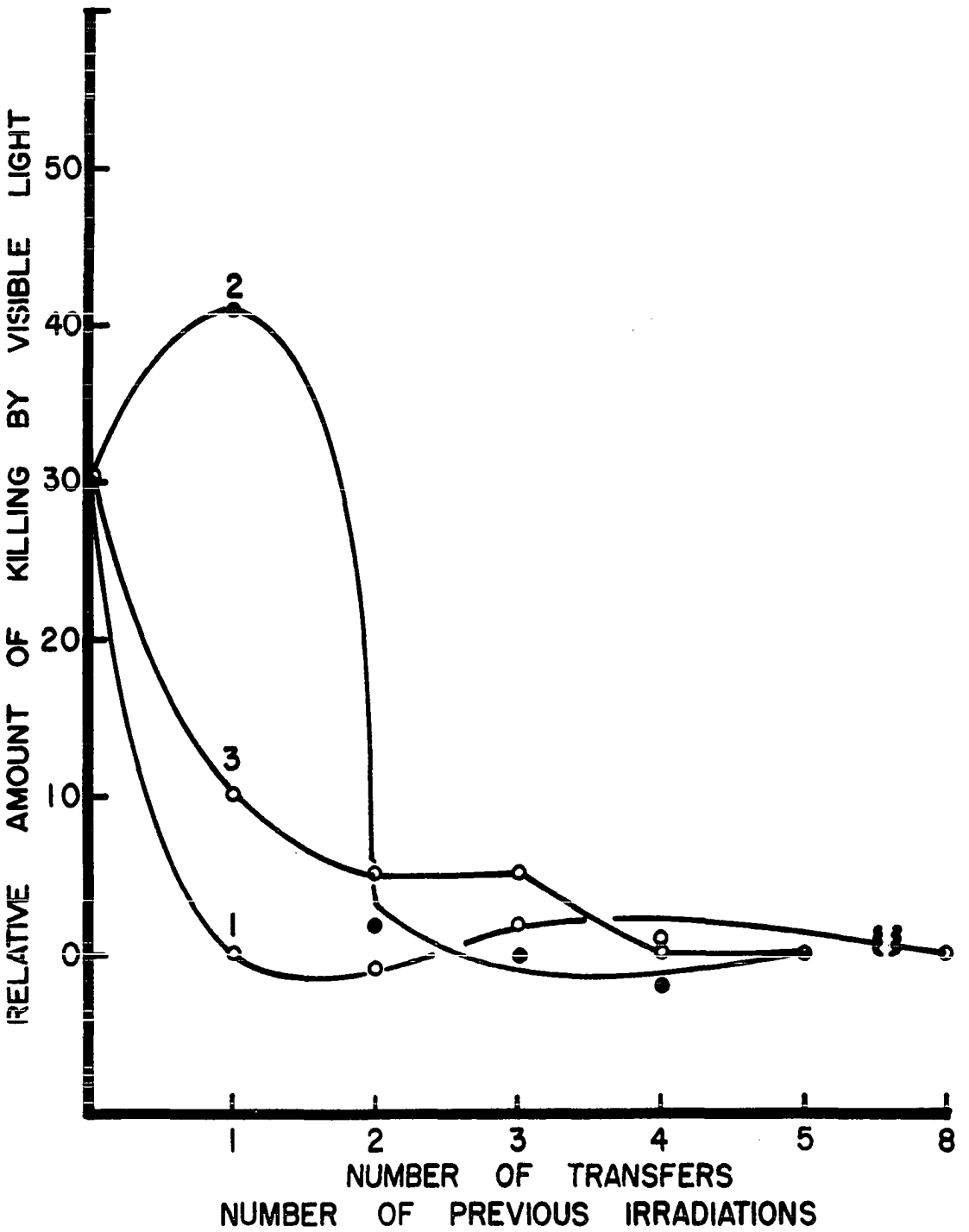

RELATIVE AMOUNT OF KILLING BY VISIBLE LIGHT
50
40
30
20
10
0
2
3
1
1
2
3
4
5
8
NUMBER OF TRANSFERS
NUMBER OF PREVIOUS IRRADIATIONS

Figure 11. The effect of growth under continuous illumination on x-ray sensitivity of a 5 hour brain heart infusion broth culture.

Curve 0. Control, no previous growth under continuous illumination.

Curve 1. One 24 hour growth period under continuous illumination.

Curve 2. Two 24 hour growth periods under continuous illumination.

Curve 4. Four 24 hour growth periods under continuous illumination.

Curve 8. Eight 24 hour growth periods under continuous illumination.

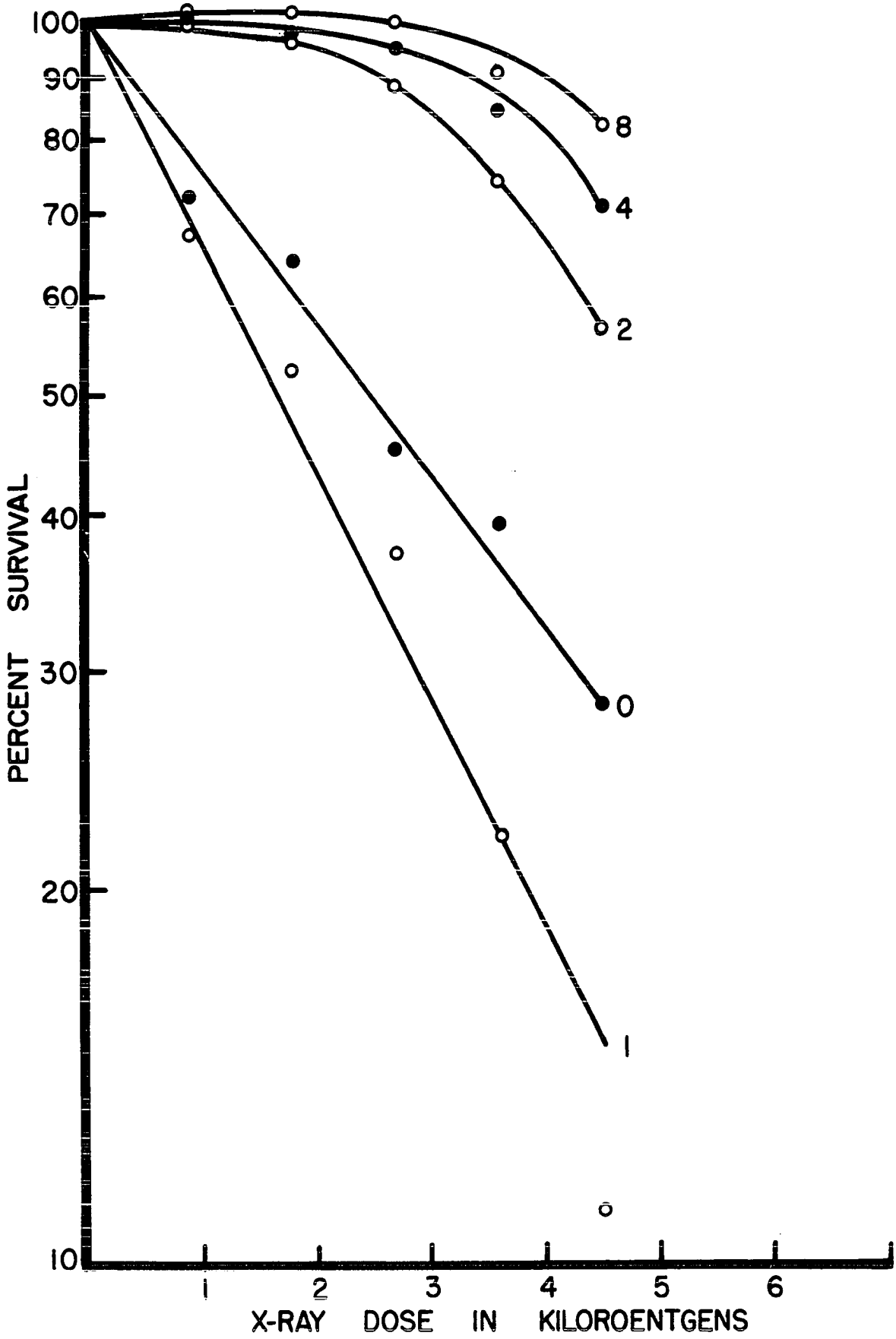

100
90
80
70
60
50
40
30
20
10
PERCENT SURVIVAL
8
4
2
0
1
1
2
3
4
5
6
X-RAY DOSE IN KILOROENTGENS

Figure 12. The effect of visible light selection on x-ray sensitivity of a 5 hour brain heart infusion broth culture.

Curve 0. No previous exposure to visible light irradiations.

Curve 1. One previous exposure to a dose of 3.5×10^5 ergs/mm^2.

Curve 2. Two previous exposures to visible light.

Curve 3. Three previous exposures to visible light.

Curve 5. Five previous exposures to visible light.

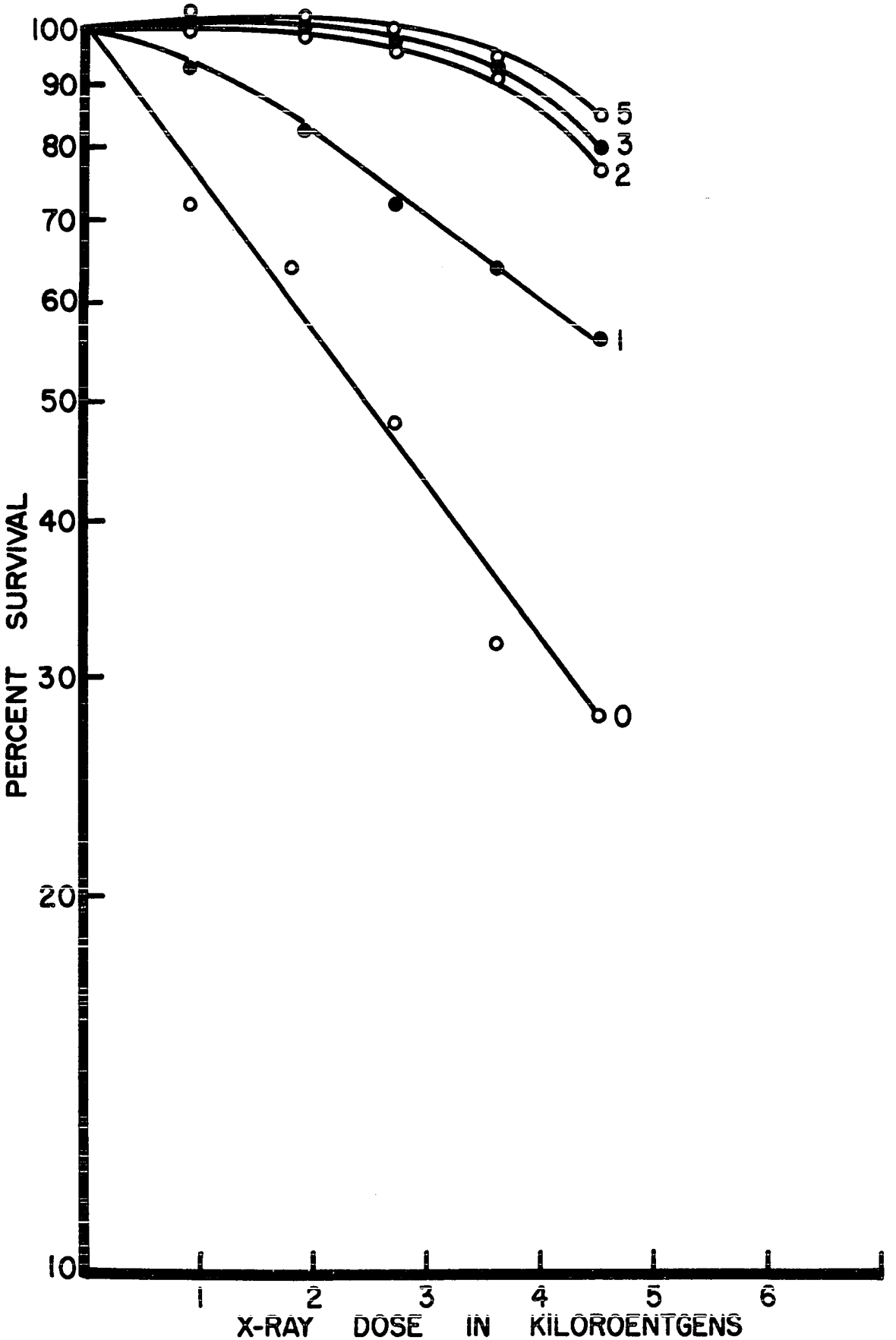
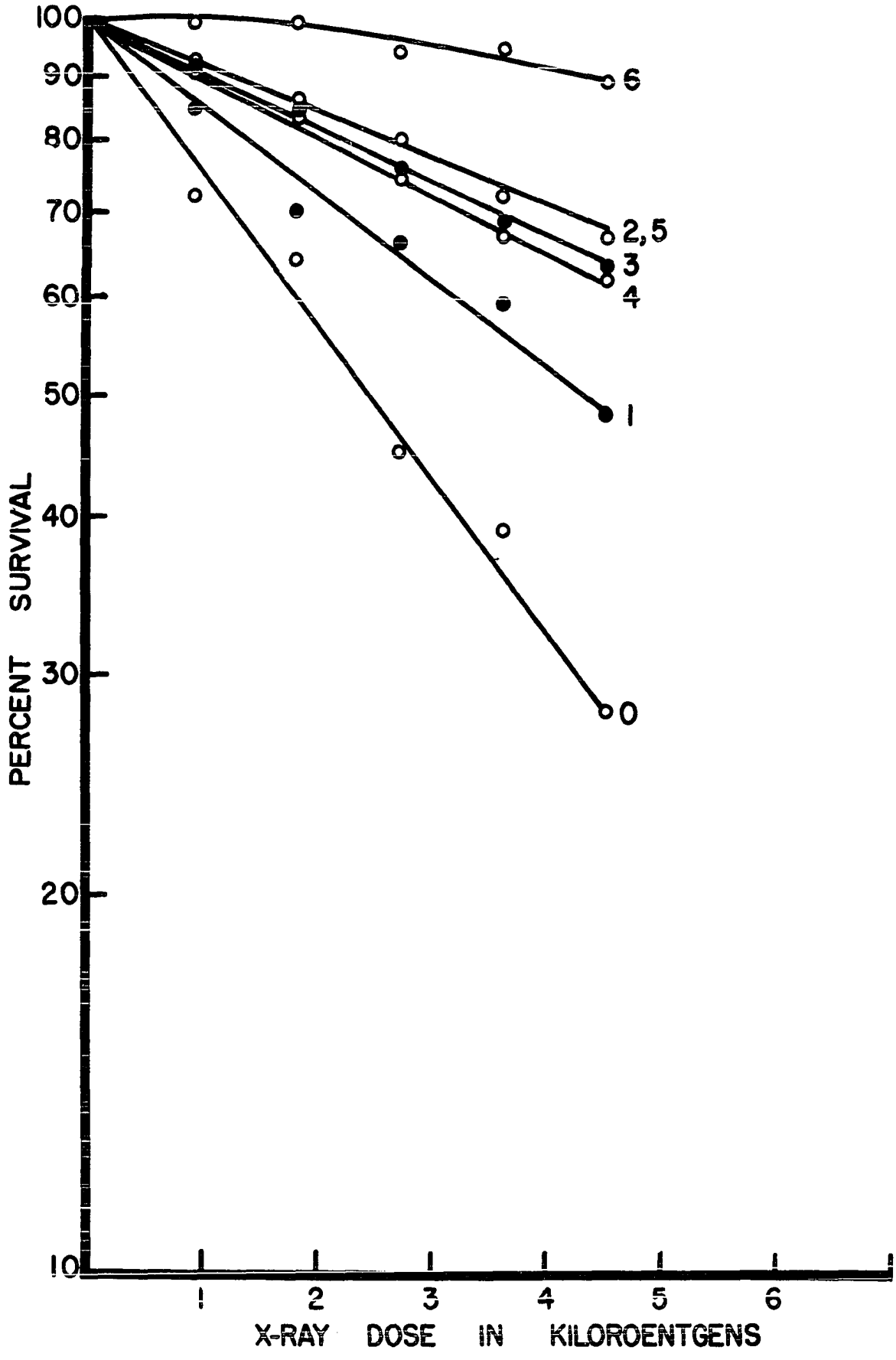

100
90
80
70
60
50
40
30
20
10
PERCENT SURVIVAL
5
3
2
1
0
1
2
3
4
5
6
X-RAY DOSE IN KILOROENTGENS

Figure 13. The effect of x-ray selection on x-ray sensitivity of a 5 hour brain heart infusion broth culture.

Curve 0. No previous exposures to a dose of 4500 r.
Curve 1. One previous x-ray exposure.
Curve 2. Two previous x-ray exposures.
Curve 3. Three previous x-ray exposures.
Curve 4. Four previous x-ray exposures.
Curve 5. Five previous x-ray exposures.
Curve 6. Six previous x-ray exposures.


100
90
80
70
60
50
40
30
20
10
PERCENT SURVIVAL
6
2,5
3
4
1
0
1
2
3
4
5
6
X-RAY DOSE IN KILOROENTGENS

of growth (Figures 1 and 2). It is also at this time that the control cells are more sensitive to x-ray damage. Stapleton (1955) demonstrated in E. coli that x-ray resistance was maximum in the lag and stationary phase with maximum sensitivity at the end of the log phase. The most obvious condition of the cells in the log phase is their state of active cell division. Transfer of cells in this phase to fresh medium eliminates the typical lag phase seen when stationary cells are transferred. One characteristic of lag phase and stationary phase cells which might account for the increased x-ray resistance is the growth lag which accompanies these cells after irradiation and subsequent plating on fresh medium. In view of the data on recovery from x-ray damage being dependant, to some extent, on the rate at which cells are allowed to undergo cell division (Adler and Hardigree, 1965) the question arises as to whether photoprotection against x-ray might be a means of altering the growth or division rate to allow more time for cell recovery. It has been shown that light in the range 350-490 mμ causes a retarded growth phase in E. coli (Hollaender, 1943). This has been extended to provide a mechanism for photoprotection against ultraviolet damage in E. coli (Jagger et al., 1964). The light used in these experiments also causes some type of damage as evidenced by the photolethal effect. In addition, the effective portion of the spectrum in photoprotection against x-ray falls in this approximate range (400-450 mμ) (Raizen, 1963).

In order to determine any effect on growth, 5 hour cells were harvested, washed, irradiated and placed in brain heart infusion broth and the changes in turbidity determined. The washed cells were stored at room temperature for approximately 30 minutes before irradiation in order to alleviate cell death due to the visible light. Jagger et al. (1964) reported that a photolethal effect on E. coli was prevented by starvation for one hour, a result essentially duplicated on S. aureus (Savage, unpublished data). Variations of the cell age, incubation temperature, visible light dose rate, and x-ray dose rate compatible with previous results were made, and the effect of these variables on growth of irradiated cells was determined. In addition to turbidimetric measurements, plate counts were used to determine percentage survivors and growth rate after the various irradiations.

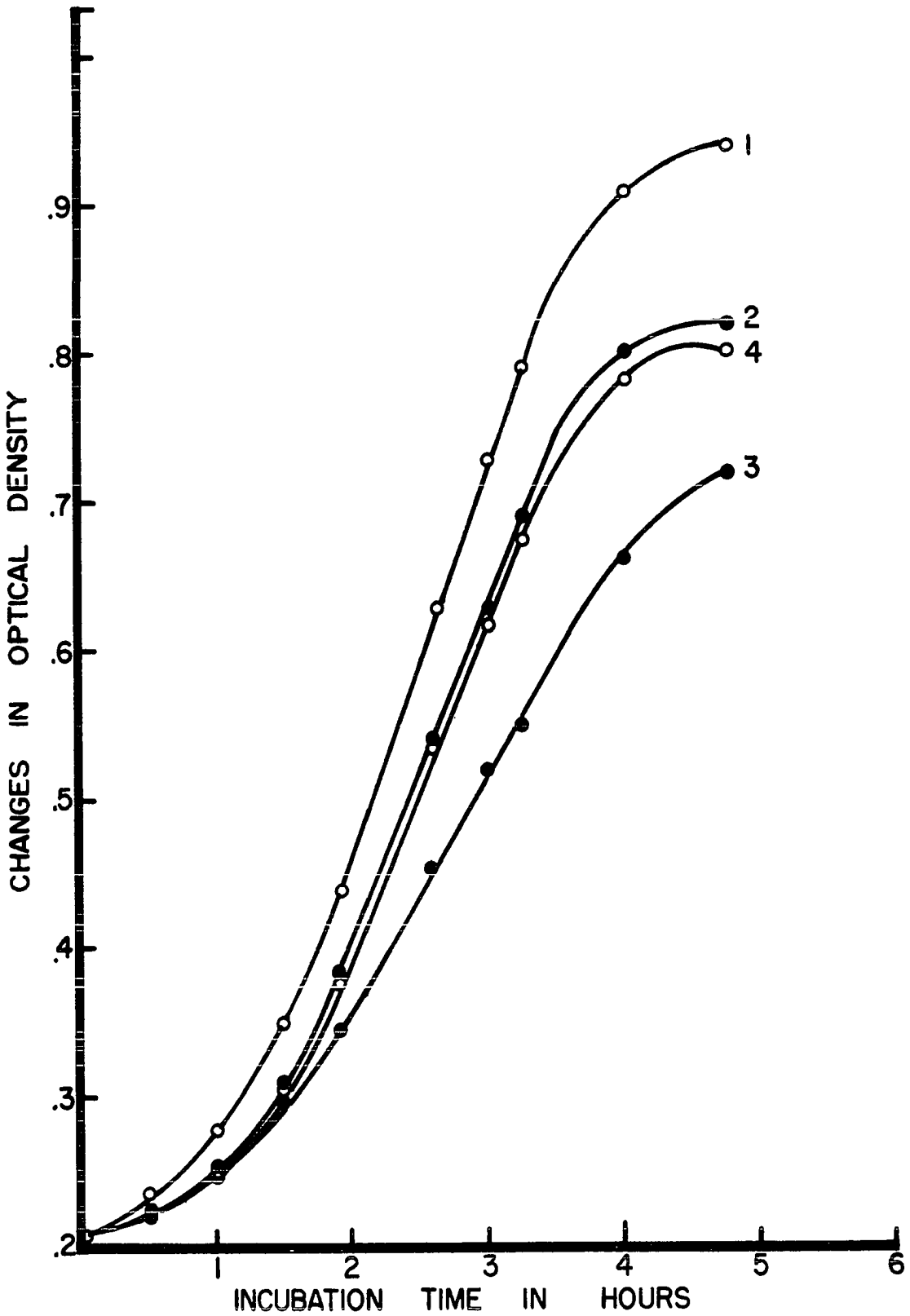
An induction of a growth delay by visible light is evident from the data presented in Figure 14. This represents a single, typical experiment consisting of control cells (1), illuminated cells (2), photoprotected cells (3), and x-irradiated cells (4). The apparent growth delay of the x-irradiated sample is dispelled when the percentage survivors of this treatment are considered. Approximately 53 % of the x-irradiated cells were able to form visible colonies in comparison to 74 % survivors for the photoprotected cells and 96 % for the visible irradiated sample. The generation time of cells inoculated into fresh media after exposure

Figure 14. The effect of visible light, visible light and x-ray, and x-ray on the growth rate.

Curve 1. Control, no irradiations.
Curve 2. Irradiated with visible light.
Curve 3. Irradiated with visible light and x-ray.
Curve 4. Irradiated with x-ray.

Visible light dose rate was 400 ergs/sec/mm^2 for a total dose of 3.5 X 10^5 ergs/mm^2. X-ray dose rate was 300 r/min for a total dose of 4500 r.

The cells used for Figures 14, 15, 16, 18, and 19 were from a 5 hour brain heart infusion broth culture. The incubation temperature was 37 C for Figures 14, 15, 16, 17, and 19.


CHANGES IN OPTICAL DENSITY
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1
2
3
4
5
6
INCUBATION TIME IN HOURS
1
2
4
3

to visible and x-irradiation was determined by plate counts. From 0 to 90 minutes the generation time was found to be 82 minutes for the control cells, 119 minutes for the cells exposed to visible light, 100 minutes for the cells exposed to visible light and x-ray, and 67 minutes for x-irradiated cells. Essentially the same pattern was found in early log phase (120-180 minutes). The generation time of control cells in this phase was 47 minutes compared to 74 minutes for the visible light exposed cells, 63 minutes for the photoprotected cells, and 38 minutes for x-irradiated cells. Although this growth delay is short in comparison to the time involved in such recovery processes as liquid holding recovery (Roberts and Aldous, 1949) or reduced incubation temperatures (Stapleton, 1960), it is sufficient to allow a considerable amount of recovery in actively metabolizing cells.

Since this initial experiment yielded results showing that visible light induced a division delay, several previously obtained results were selected to determine if a definite correlation might exist. Howell (unpublished data) found that exposure of the cells to visible light at a reduced dose rate for longer periods of time resulted in almost a two-fold increase in the amount of photoprotection. A correlation, therefore, of a visible light dose rate effect on growth delay with this data would support the involvement of increased repair as the mechanism in photoprotection.

The results in Figure 15 show that cells illuminated at a dose rate of 200 ergs/sec/mm^2 for 30 minutes have a slower growth rate than cells illuminated at 400 ergs/sec/mm^2 for 15 minutes. The amount of delay for the lower dose rate was almost twice that of the higher dose rate when compared to the control. The period of time required for the optical density of the control to double was 120 minutes in comparison to 135 minutes for the sample receiving the higher dose rate and 155 minutes for the sample receiving the lower dose rate. The survivors of these samples were 93 % at the higher dose rate and 98 % at the lower dose rate.

Photoprotection has also been reported to be x-ray dose rate dependent (Clark and Frady, 1961). Dose rate dependency has been considered as evidence for a repair mechanism (Stapleton, 1960). The effect of visible light on growth of cells irradiated at 3000 r/min was, therefore, determined, and the results compared with results obtained from cells exposed to a dose rate of 300 r/min. The same total x-ray dose (4500 r) was given both samples (Fig. 16). These results show that the growth rate of the cells irradiated at 3000 r/min is considerably reduced when compared to those irradiated at 300 r/min. Also, the visible light induced delay is nullified so that the growth rate of cells exposed to visible light prior to x-ray is approximately the same as that of cells not previously illuminated.

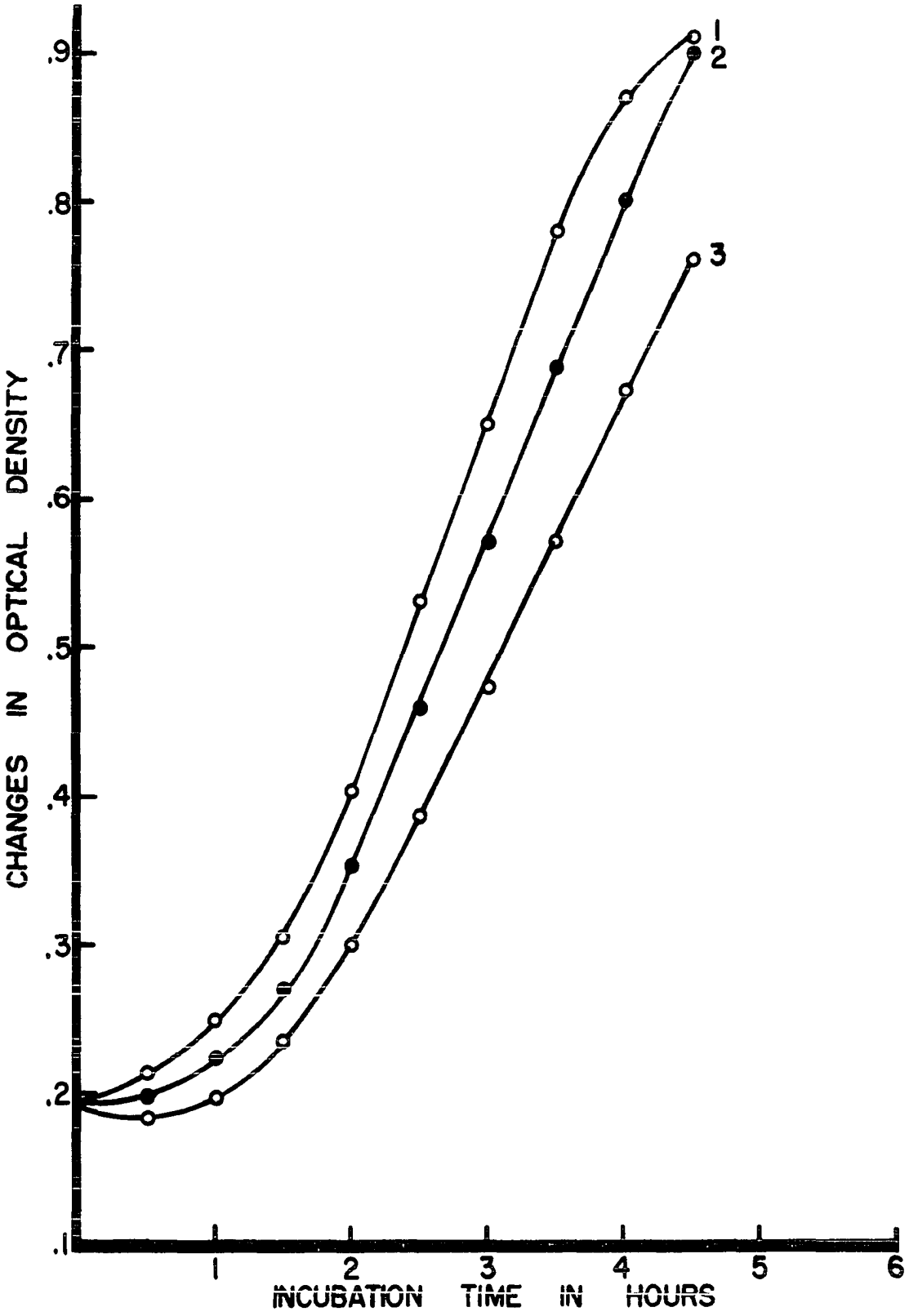
Figure 17 presents more evidence that the growth delay induced by visible light is involved in photoprotection.

Figure 15. The visible light dose rate effect on growth rate.

Curve 1. Control, no visible light.

Curve 2. Sample exposed to dose rate of 400 ergs/ sec/mm^2 for 15 minutes.

Curve 3. Sample exposed to dose rate of 200 ergs/ sec/mm^2 for 30 minutes.


CHANGES IN OPTICAL DENSITY
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4
5
6
INCUBATION TIME IN HOURS
1
2
3

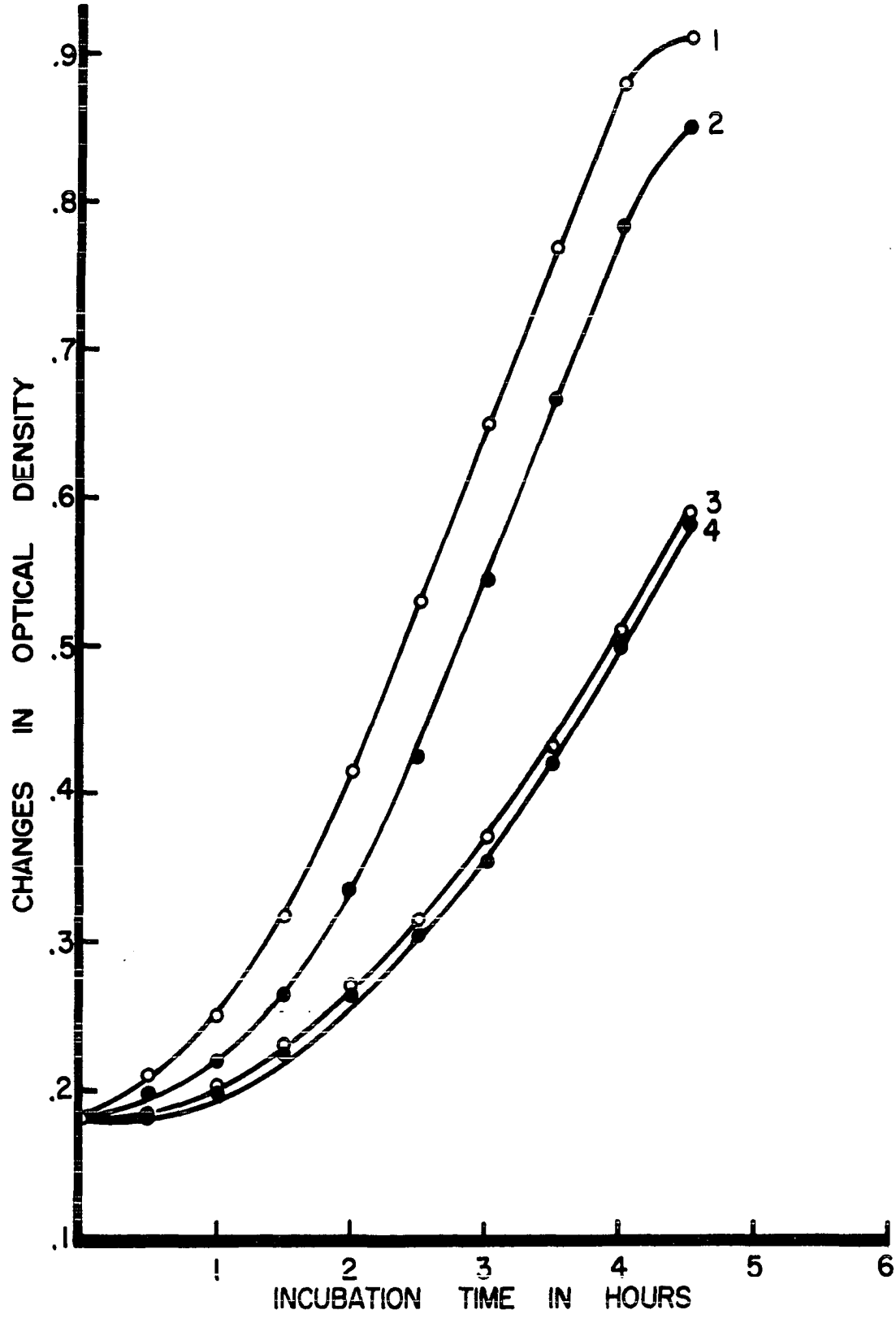
Figure 16. The x-ray dose rate effect on growth rate.

Curve 1. Control, no irradiations.

Curve 2. Sample irradiated at 300 r/min for 15 minutes.

Curve 3. Sample irradiated at 3000 r/min for 1½ minutes.

Curve 4. Sample irradiated with visible light prior to x-irradiation at 3000 r/min for 1½ minutes.


CHANGES IN OPTICAL DENSITY
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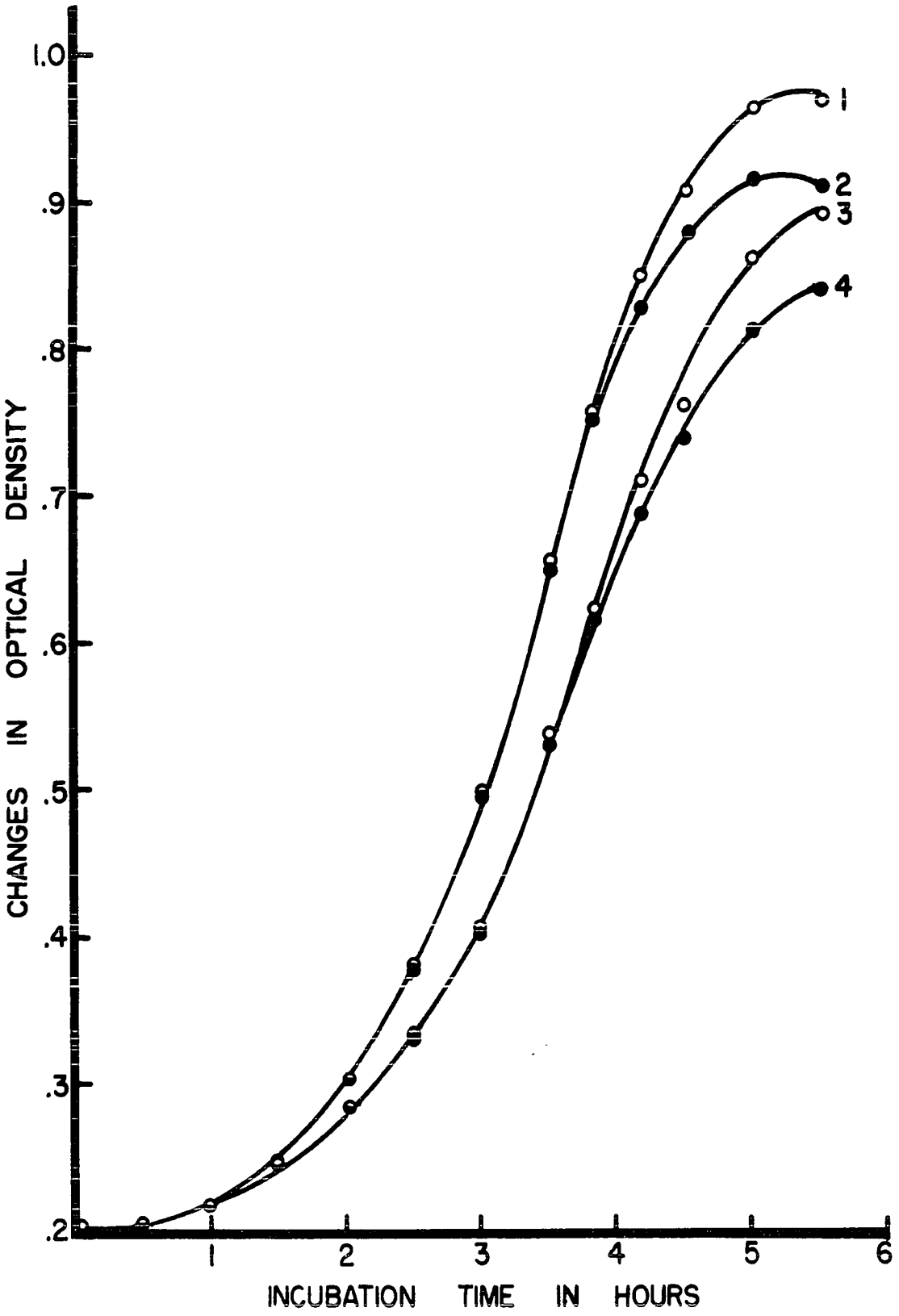
4
5
6
INCUBATION TIME IN HOURS
1
2
3
4

Figure 17. The effect of light, light and x-ray, and x-ray on the growth rate of 24 hour cells (0 time).

Curve 1. Control, no irradiations.
Curve 2. Sample irradiated with visible light.
Curve 3. Sample irradiated with visible light and x-ray.
Curve 4. Sample irradiated with x-ray.


1.0
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CHANGES IN OPTICAL DENSITY
1
2
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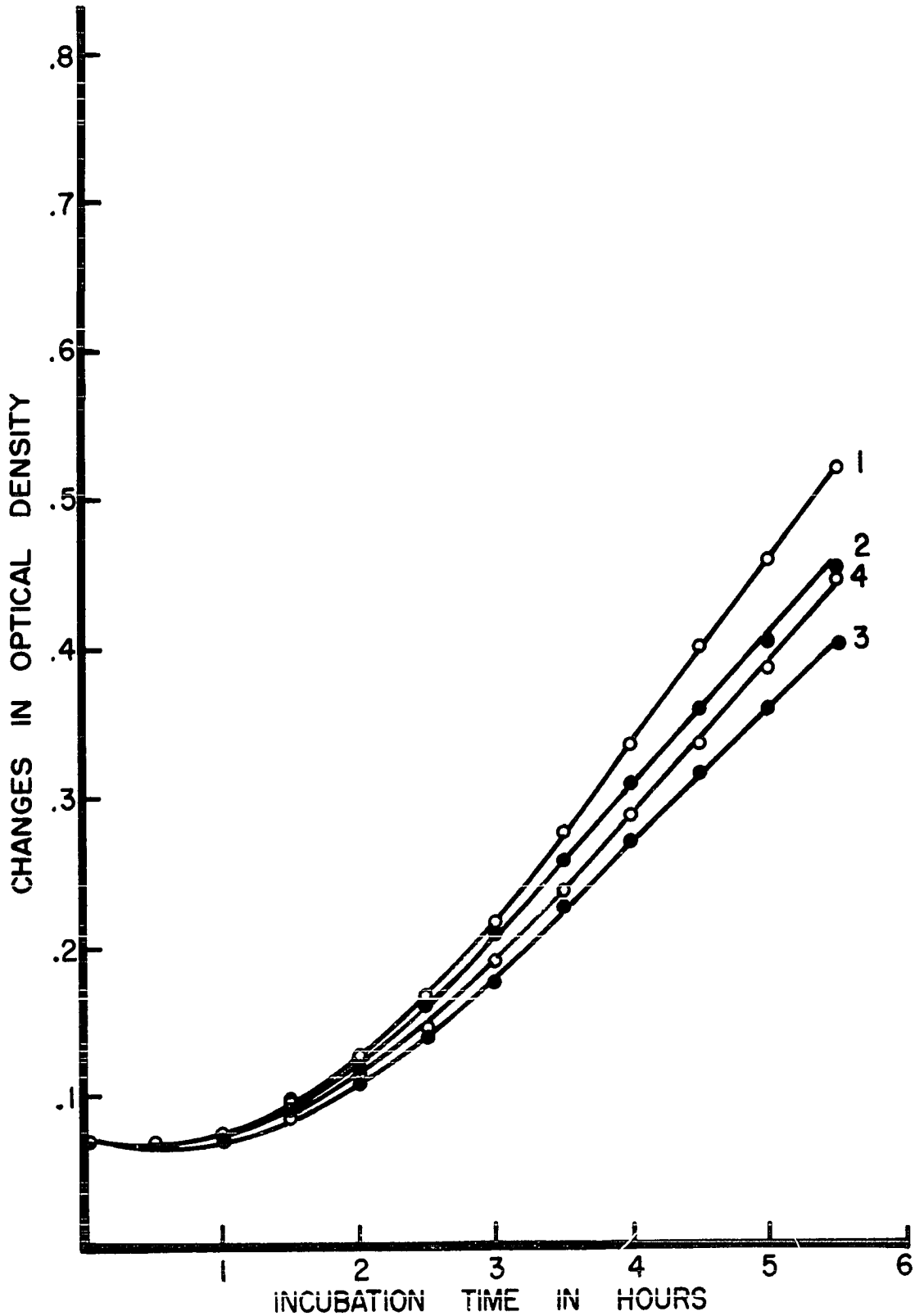
4
1
2
3
4
5
6
INCUBATION TIME IN HOURS

Cells harvested from an agar slant (24 hour) and inoculated into fresh broth (0 time) rarely show photoprotection and more often show photosensitization. There may be two factors involved. First, there is an increased lag phase of growth of the cells due to their adaptation from a stale to a fresh medium in comparison to log phase cells. Therefore, there is more time for all of the cells to recover from x-ray damage before cell division. Secondly, the light may not have any effect on cells in this particular physiological state. The answer appears to be the latter possibility since the growth rates of the control and visible light irradiated samples are the same, and the photoprotected and x-rayed sample growth rates are the same. No photoprotection was obtained with these cells.

Another instance in which a reduced overall growth rate might be instrumental in overshadowing any photoprotective effect is found in cells incubated at 29 C (Fig. 3) rather than 37 C (Fig. 1). Cells incubated at this lower temperature do not show any photoprotection. Growth studies on log phase cultures grown at 29 C (Fig. 18) do not substantiate this suggestion. The pattern of the curves is identical with the curves at 37 C (Fig. 15), but the growth rates are greatly reduced. This lack of any photoprotection at this reduced incubation temperature may be reflected in a decreased activity of the repair mechanism so that, although there is a slower growth rate, the apparatus necessary for repair

Figure 18. The effect of light, light and x-ray, and x-ray on the growth rate of cells incubated at 29 C.

Curve 1. Control, no irradiations.
Curve 2. Sample irradiated with visible light.
Curve 3. Sample irradiated with visible light and x-ray.
Curve 4. Sample irradiated with x-ray.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CHANGES IN OPTICAL DENSITY
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4
3
1
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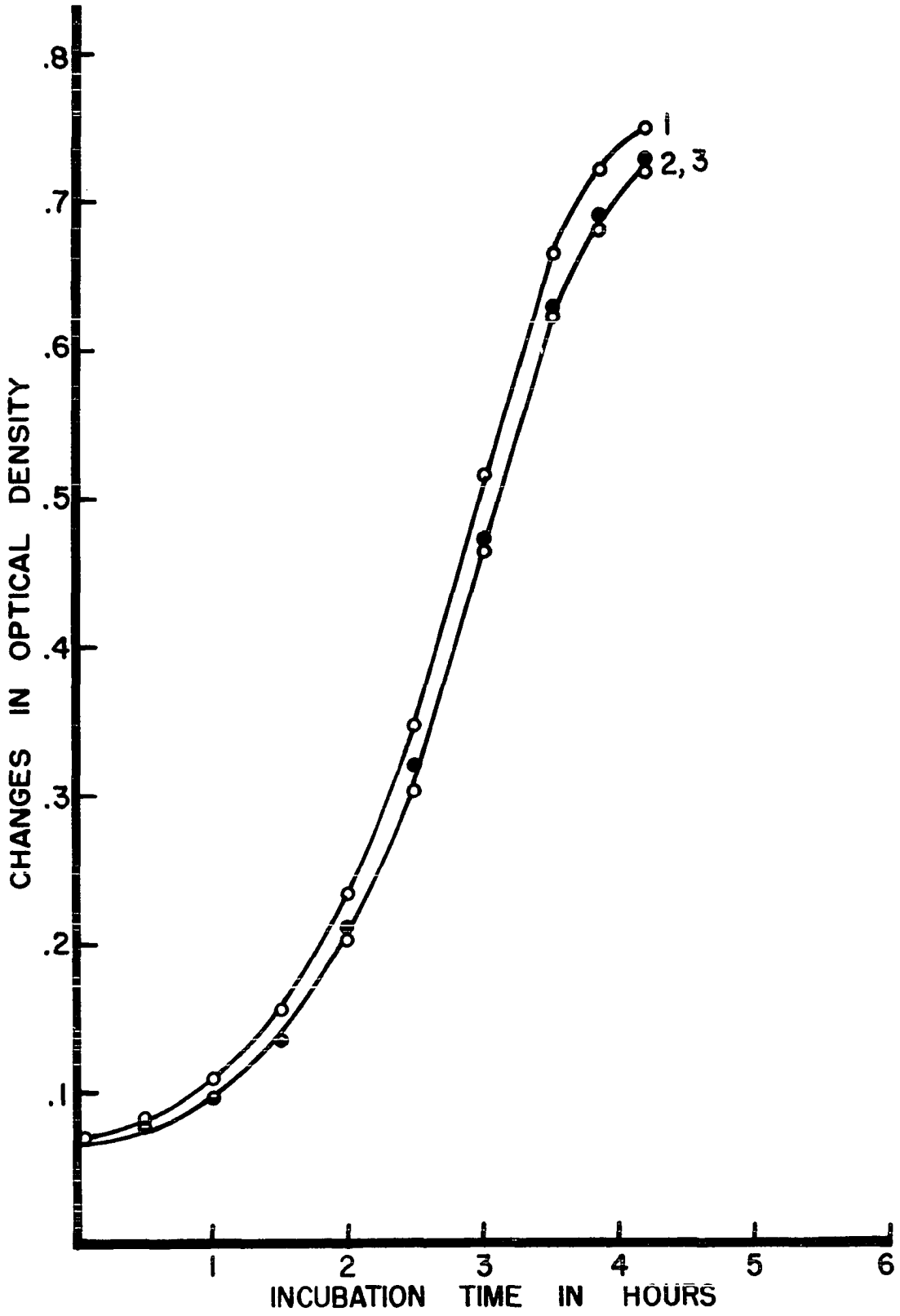
4
5
6
INCUBATION TIME IN HOURS

sould also be slower, and fewer cells would survive the x-irradiations. That this is a possibility may be deduced from the apparent increase in the overall x-ray sensitivity as a result of these conditions. The requirements for repair in different organisms, even closely related strains of E. coli (Adler and Hardigree, 1965), are, in many cases, just opposite. For example, E. coli B cells recover more readily from irradiation damage at temperatures above the growth optimum (Anderson, 1951); E. coli B/r recovers at temperatures below the growth optimum (Stapleton, 1955).

If the effect of visible light which results in protection against x-ray damage is, in effect, a means of allowing more time for cell repair, it seems logical that the light could be administered either before, during, or after the x-ray. The easier of the latter two possibilities is to expose previously x-rayed cells to visible light (Fig. 19). Since there have been no unequivocal reports of photoreactivation of x-ray damage, this post-x-ray visible light exposure should not give misleading results. Cells of various ages were tested for reactivation of x-ray damage by light, but no reactivation was found. In fact, exposure of x-rayed cells to visible light resulted in some killing by the light. The percent survivals for a 5 hour sample were 70 % for the control (stirred only after x-ray) and 51 % for the sample exposed to visible light after x-ray. These survivals were determined from the x-ray survivals

Figure 19. The effect of x-ray and x-ray followed by light on the growth rate.

Curve 1. Control, no irradiations.
Curve 2. Sample irradiated with x-ray followed by light.
Curve 3. Sample irradiated with x-ray.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CHANGES IN OPTICAL DENSITY
1
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5
6
INCUBATION TIME IN HOURS

which represented 100 %. The data in Figure 19 shows that the visible light does not alter the growth rate of previously x-irradiated cells. Nor does it afford any protection. These results are consistent with a correlation of a light-induced growth delay being necessary for photoprotection. The reason the visible light is ineffective when applied after the x-ray is unknown. However, this is a possible explanation for the inability to demonstrate photoreactivation of x-ray damage. Since there is no growth delay of cells treated in this manner, there is no more time for the illuminated cells to repair x-ray damage.

CHAPTER IV

DISCUSSION

One of the best understood recovery processes is photoreactivation of ultraviolet damage (see review by Rupert and Harm, 1966). Dark-recovery phenomena such as host cell reactivation (Garen and Zinder, 1955), liquid holding recovery (Roberts and Aldous, 1949) u or v gene reactivation (Streisinger, 1956) are less well characterized but are also thought to act enzymatically and in some cases overlap photoreactivation (Harm, 1963; Metzer, 1963). Studies of these recovery phenomena have increased considerably the understanding of the lesions produced by ultraviolet light.

The understanding of x-ray induced lesions has not progressed as rapidly as that of ultraviolet lesions. However, a few recent studies have suggested that there are enzyme-mediated recovery systems (Davies and Evans, 1966).

Environmental conditions following x-irradiation such as temperature, media, and oxygen tension are some of the more widely studied means of altering x-ray lethality (Stapleton, 1960; Hodgkins and Alper, 1963). In addition, Howell (1966) found that the manner in which the organism

is treated prior to the x-irradiation is of equal importance as a determinant of x-ray sensitivity.

In view of the many ways in which the radiosensitivity of an organism may be changed, it becomes very difficult to reach conclusions regarding any experimental evidence which is not subject to several other interpretations. Also, the lack of a single, definite biological lesion produced by x-irradiation and the wide range of possibilities adds to the problem.

Variations of x-ray sensitivity and photoprotection indicated in the evidence presented here may, therefore, be the result of multiple factors. Treatment of the cells in an attempt to increase the amount of photoprotection may be overshadowed by an alteration of the overall sensitivity of the control cells. It seems that the extreme variability of early results on photoprotection was due primarily to the age of the cells being used. Use of stationary phase cultures, in this respect, would likely result in more consistent x-ray sensitivities, but the photoprotective phenomenon, which apparently depends on the physiological state of the organism more than radiation sensitivity, would be more variable. Cultivation of the organism on different types of media would be expected to alter its x-ray sensitivity as well as its photoprotective capacity. But an apparent increase in the amount of protection afforded by visible light is nullified if the resistance of the control cells

increases. This type of reaction was seen in the study on the effects of the two different media. The same result is seen in the case of different incubation temperatures. By altering the metabolism of the cell with these factors, the normal ability of the cell to recover from x-ray is altered. The total metabolism of the cell incubated at lower temperatures would have a decreased rate, cell division is slowed, and a greater recovery might be expected. However, this slower metabolism is not always conducive to recovery as evidenced by the effect of higher temperatures on recovery and the effects of minimal versus complete medium (Stapleton, 1960; Alper and Gillies, 1960). A critical point seems to be the rate at which cells divide after receiving x-ray damage. Recovery may be hampered if the cells are held in non-nutrient or minimal medium if their synthetic capabilities are somewhat limited, or some portion of a synthetic pathway is damaged and a lack of essential substances in the medium would prevent a bypass of the damaged part.

An alteration of the conditions after x-irradiation, in comparison to preirradiation, would also put a strain on the cell in regard to its ability to use available energy to repair x-ray damage. An extra amount of cellular activity would be directed toward adaptation to a new set of growth conditions with the result that normal recovery processes would suffer. This may be an explanation for the results on the increase in x-ray sensitivity of cells cultured

anaerobically before x-irradiation and aerobically after, although x-irradiation was done under conditions which normally would increase x-ray resistance. This would be expected to alter photoprotection also, but this does not appear to be the critical point in this regard. Demonstration of photoprotection under conditions mentioned above with the cells irradiated under air implicates the manner of x-irradiation as the critical factor in photoprotection, and this is supported by experimental results on aerobically grown, but anoxically x-irradiated cells. This indicates that photoprotection acts on oxygen dependent x-ray damage. Normally, damage to the genetic material is not considered to result from the indirect action of x-rays, nor is repair usually considered effective on non-genetic damage. However, a few recent reports have contained contrary results. It was observed that starvation of Pseudomonas for 48 hours after irradiation eliminated damage that was oxygen dependent but had no effect on oxygen independent damage (Gray, 1961). On the other hand, it has been found that rescuing treatments are more effective when cells are irradiated anoxically rather than aerobically (Davies and Evans, 1966). Results of this study were interpreted to mean that the overall sensitivity is a combination of sensitivities of more than one target, and any postirradiation modification will act differentially on these targets. The authors concluded that ultraviolet damage and x-ray damage produced anoxically are

similar and presumably act on nucleic acid, whereas the target for oxygen dependent damage of x-ray is different. Studies on the effect of postirradiation growth on acriflavine containing media (Davies and Evans, 1966) support this idea. Cells exposed to ultraviolet were most sensitive, anoxically x-irradiated cells next, and aerobically x-irradiated cells least sensitive, indicating that DNA under aerobic x-irradiation is a less important target than under anoxic conditions. However, an alternative explanation is that different lesions are formed in the DNA under aerobic and anoxic x-irradiations. It may be surmised that the damage caused by x-ray under aerobic conditions is amenable by a repair mechanism although it may not be the same type of repair seen with x-irradiations under anoxic conditions.

The results of the experiments on continuous illumination and selection of survivors rule out the possibility of selection as being the explanation for photoprotection. Although selection of more resistant populations is indicated by the general increase of x-ray and visible light resistance, there is no correlation between an increased light sensitivity and decreased photoprotection except in the case of selection of resistant cells by visible light. This result is contradicted, however, by the lowered visible light sensitivity of the continuously illuminated and x-ray selected cells and the increase of photoprotection with its more gradual decline as a result of overall resistance of the

population. That there is a change in the apparent kinetics of inactivation by x-ray of the treated cells seems evident from the change in the shape of survival curve from exponential to sigmoidal. Previous interpretation of a sigmoidal curve was that it represented a multitarget or multihit phenomenon (Hutchinson and Pollard, 1961). Cell clumping will also give a sigmoidal survival curve. Recently, Haynes (1964) suggested that in light of evidence on the importance of repair mechanisms, an alternative interpretation of survival curves may be possible in some instances. He suggested that cells may have only a certain capacity for repair, and this may be inhibited or saturated at high doses. That is, the initial slope of a curve may reflect a sensitivity with an active repair mechanism and the final slope the sensitivity with an inhibited repair mechanism. This suggestion may be applicable to the changes observed in sensitivity of the cells which were constantly illuminated and selected. The x-ray resistance of the cells may well be due to selection in which the resultant culture is resistant by virtue of a more efficient repair system.

From the results presented here, one effect of visible light is to induce a growth delay. According to previous results on repair, this would allow a larger percentage to recover from the x-ray damage. Jagger et al. (1964) found a visible light-induced growth and division delay which they offer as a mechanism for photoprotection

of ultraviolet damage. However, the wavelengths of protecting light they used (338 mμ) were considerably shorter than those reported for protection against x-ray damage (400-450 mμ). Correlations of growth delay and photoprotection are seen in many of the results presented. For example, this correlation is evident in the experiments on visible light dose rate dependency, x-ray dose rate dependency, incubation temperature effect, and cell age. The fact that cells incubated at 29 C do show a growth delay but no photoprotection does not detract from the repair idea. If the cells are capable of only a limited amount of repair, the extended lag phase of all the cells might be sufficient to allow maximum recovery. Also, the repair mechanism may be less effective at this temperature. This latter possibility seems more feasible since the cells were more sensitive to x-ray when incubated at this temperature. The reason for the lack of a decreased growth of cells subjected to visible light after x-ray is obscure. It might be that the same site which is responsible for the visible light-induced delay is also affected by x-ray, and once it has been damaged by x-ray it no longer is susceptible to the visible light. This is not indicated from the results, however, since some of the x-rayed cells are still susceptible to the lethal effect of visible light. These light sensitive cells may have escaped damage by the x-ray. If so, a decreased growth rate should occur with other undamaged cells in the culture.

On the other hand, the light may be acting synergistically with non-lethal x-ray damage to kill the cells, so that the light sensitive fraction after x-irradiation is entirely different from the light sensitive fraction before x-ray.

Although the observed growth delay induced by visible light is not a large effect, it may still offer an explanation for photoprotection against x-ray damage. The amount of protection against x-ray by visible light is not large as in photoreactivation. And a considerable amount of repair could occur in the small amount of extra time before cell division.

The mechanism whereby the visible light induces the growth delay is still unknown. Jagger et al. (1964) proposed the quinones of the hydrogen transport system as the chromophore. If the action spectrum for photoprotection against x-ray is correct, these compounds are essentially ruled out as a possibility. Any attempt to say what the chromophore is, at this time, would be mere speculation.

CHAPTER V

SUMMARY AND CONCLUSIONS

The effect of various environmental conditions on photoprotection against x-ray damage was determined. Results from these experiments indicated that maximal photoprotection resulted from the use of a culture in log phase. The amount of photoprotection was not affected by culturing the organism on brain heart infusion or nutrient broth. A reduction of the incubation temperature before and after irradiation from 37 C to 29 C resulted in a loss of any photoprotective effect. Anaerobic incubation had no effect other than a possible reduction in the amount of photoprotection, but x-irradiation under anoxic conditions completely eliminated the protective effect. This was interpreted to mean that photoprotection acts on x-ray damage which is oxygen dependent.

The possibility that visible light was selecting for x-ray resistant cells was also investigated. Growth of the organism under constant illumination, selection of visible light resistant cells, and selection of x-ray resistant cells gave results indicative of a mixed population. However, no correlation of visible light sensitivity and photoprotection

was found. It was concluded, therefore, that photoprotection was a real effect.

Growth of the organism in broth following visible and x-irradiations, singly and in combination, indicated that visible light induced a small growth delay. X-irradiation, on the other hand, increased the growth rate so that a comparison of growth rates of photoprotected and x-irradiated cells indicated a significant difference. A correlation of this growth delay with several previously observed characteristics of photoprotection suggested that this delay might allow more time for repair by normal cell recovery processes. An attempt was made to explain some of the observed results on photoprotection and x-ray sensitivity on the basis of this recovery process.

BIBLIOGRAPHY

Adler, Howard I., and Alice A. Hardigree. 1965. Postirradiation growth, division, and recovery in bacteria. Rad. Res. 25:92-102.

Alper, T., and N. E. Gillies. 1960. The relationship between growth and survival after irradiation of Escherichia coli strain B and two resistant mutants. J. Gen. Microbiol. 22:113-128.

Anderson, E. H. 1951. Heat reactivation of ultraviolet inactivated bacteria. J. Bacteriol. 61:389-394.

Blum, H. F., J. C. Robinson, and G. M. Loos. 1951. The loci of action of ultraviolet and x-radiation and of photorecovery in the egg and sperm of the sea urchin, Arbacia punctulata. J. Gen. Physiol. 35:323-342.

Cantelmo, P. 1951. Prevention et negativation par la lumiere visible de l'induction lysogenique aux ultraviolets. Compt. Rend. Soc. Biol. 145:1882-1884.

Clark, J. B., and Janice Frady. 1951. Photoprotection against x-ray inactivation in Nocardia carallina. J. Bacteriol. 81:524-526.

Dulbecco, R. 1950. Experiments on photoreactivation of bacteriophages inactivated with ultraviolet radiation. J. Bacteriol. 59:329-347.

Dulbecco, R., and J. J. Weigle. 1952. Inhibition of bacteriophage development in bacteria illuminated with visible light. Experientia. 8:386-387.

Davies, D. R., and H. J. Evans. 1966. The role of genetic damage in radiation-induced cell lethality, p 243-353. In Leroy G. Augenstein, Ronald Mason, and Max Zelle (ed.), Advances in Radiation Biology. Volume II. Academic Press, New York and London.

Eldjarn, Lorentz, and Alexander Pihl. 1960. Mechanisms of protective and sensitizing action, p 231-296. In

Maurice Errera and Arne Forssberg (ed.), Mechanisms in Radiobiology. Volume II. Academic Press, New York and London.

Garen, Alan, and Norton D. Zinder. 1955. Radiological evidence for partial genetic homology between bacteriophage and host bacteria. Virology. 1:347-376.

Giese, A. C., C. L. Brandt, R. Iverson, and P. H. Wells. 1952. Photoreactivation in Colpidium colpoda. Biol. Bull. 103:336-344.

Giese, A. C., C. L. Brandt, C. Jacobson, D. C. Shepard, and R. T. Sanders. 1954. The effects of starvation on photoreactivation in Colpidium colpoda. Physiol. Zool. 27:71-78.

Gray, L. H. 1961. Mechanisms involved in the initiation of radiobiological damage in aerobic and anaerobic systems, p 21-44. In R. J. C. Harris (ed.), The Initial Effects of Ionizing Radiations on Cells. Academic Press, London and New York.

Harm, Walter. 1963. Repair of lethal ultraviolet damage in phage DNA, p 107-124. In F. H. Sobels (ed.), Repair from Genetic Radiation Damage. MacMillan Co., New York.

Hodgkins, Brenda, and Tikvah Alper. 1963. Anaerobic growth as a factor influencing radiosensitivity. J. Gen. Microbiol. 30:307-315.

Hollaender, A., and B. M. Duggar. 1938. Effects of sublethal doses of monochromatic ultraviolet radiation on growth properties of bacteria. J. Bacteriol. 36:17-37.

Hollaender, A., and C. W. Emmons. 1941. Wavelength dependence of mutation production in the ultraviolet with special emphasis on fungi. Cold Spring Harbor Symposia Quant. Biol. 9:179-186.

Hollaender, Alexander. 1943. Effect of long ultraviolet and short visible radiation (3500 to 4900 A) on Escherichia coli. J. Bacteriol. 46:531-541.

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

Hollaender, Alexander (ed.). 1960. Radiation Protection and Recovery. Pergamon Press. New York, Oxford, London, Paris. 392 pg.

Howell, R. D. 1966. Changes in radioresistance associated with changes in metabolism of Staphylococcus aureus. Ph. D. dissertation. University of Oklahoma, Norman.

Hutchinson, F., and E. Pollard. 1961. Target theory and radiation effects on biological molecules, p 71-92. In Maurice Errera and Arne Forssberg (ed.), Mechanisms in Radiobiology. Volume I. Academic Press, New York and London.

Jagger, John. 1958. Photoreactivation. Bacteriol. Reviews. 22:99-142.

Jagger, J., and R. S. Stafford. 1962. Biological and physical ranges of photoprotection from ultraviolet damage in microorganisms. Photochem. Photobiol. 1:245-257.

Jagger, John. 1963. Photoprotection from far ultraviolet effects in cells. Advances in Chemical Physics. 7:584-601.

Jagger, John, W. Curtis Wise, and R. S. Stafford. 1964. Delay in growth and division induced by near ultraviolet radiation in Escherichia coli and its role in photoprotection and liquid holding recovery. Photochem. Photobiol. 3:11-24.

Jones, M. F., and A. Hollaender. 1942. Effect of long ultraviolet and near visible radiation on the eggs of the nematodes, Enterobius vermicularis and Ascaris lumbricoides. J. Parasitol., Suppl. 28:17-18.

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

Kelner, A. 1949b. Photoreactivation of ultraviolet-irradiated Escherichia coli with special reference to the dose-reduction principle and to ultraviolet-induced mutation. J. Bacteriol. 59:329-347.

Kimball, R. F., and N. T. Gaither. 1951a. Photorecovery of the effects of ultraviolet radiation on Paramecium aurelia. (Abstr.) Genetics. 35:118.

Kimball, R. F., and N. T. Gaither. 1951b. The influence of light upon the action of ultraviolet on Paramecium aurelia. J. Cell. Comp. Physiol. 37:211-231.

Klein, Deana T., and Richard M. Klein. 1965. Photoprotection against genetic damage in Neurospora caused by x-rays. Photochem. Photobiol. 4:623-624.

Latarjet, R. 1951. Photorestauration apres irradiation-X chez une bacterie lysogene. Compt. Rend. Soc. Biol. 232:1713-1715.

Latarjet, R. 1954. Spontaneous and induced cell restorations after treatments with ionizing and non-ionizing radiations. Acta Radiol. 41:84-100.

Metzger, K. 1963. Overlapping action of host cell reactivation and photoreactivation in bacteria. Photochem. Photobiol. 2:435-442.

Raizen, C. E. 1963. Photoprotection against x-ray inactivation in a series of microorganisms. Master's Thesis. University of Oklahoma, Norman.

Roberts, R. B., and Elaine Aldous. 1949. Recovery from ultraviolet irradiations in Escherichia coli. J. Bacteriol. 57:363-368.

Rupert, Claude S., and Walter Harm. 1966. Reactivation after photobiological damage, p 2-75. In Leroy G. Augenstein, Ronald Mason, and Max Zelle (ed.), Advances in Radiation Biology. Volume 2. Academic Press, New York and London.

Savage, N. L., R. D. Howell, and J. B. Clark. 1964. Environmental factors affecting photoprotection against x-ray damage in Staphylococcus aureus. (Abstr.) Bacteriol. Proc. p. 39.

Skerman, V. B. D. 1959. A Guide to the Identification of the Genera of Bacteria, p 205-206. Williams and Wilkins Company. Baltimore, Maryland.

Stapleton, George E. 1955a. Variations in the sensitivity of Escherichia coli to ionizing radiations during the growth cycle. J. Bacteriol. 70:357-362.

Stapleton, George E. 1955b. The influence of pretreatments and posttreatments on bacterial inactivations by ionizing radiations. Ann. N. Y. Acad. Sci. 59: 604-618.

Stapleton, G. E. 1960. Protection and recovery in bacteria and fungi, p 87-116. In Alexander Hollaender (ed.), Radiation Protection and Recovery. Pergamon Press, New York, London, and Paris.

Streisinger, George. 1956. The genetic control of ultraviolet sensitivity levels in bacteriophages T2 and T4. Virology. 2:1-12.

Tobin, J. O. 1953. Some observations on the effect of x-rays on the behavior of a temperate phage in two strains of Pseudomonas pyocyanea. Brit. J. Exptl. Pathol. 34:635-646.

Watson, J. D. 1950. The properties of x-ray-inactivated bacteriophage. I. Inactivation by direct effects. J. Bacteriol. 60:697-718.

Watson, J. D. 1952. The properties of x-ray-inactivated bacteriophage. II. Inactivation by indirect effects. J. Bacteriol. 63:473-485.

Weatherwax, R. S. 1956. Desensitization of Escherichia coli to ultraviolet light. J. Bacteriol. 72:124-125.