ULTRAVIOLET-INDUCED ABNORMAL GROWTH IN ESCHERICHIA COLI; THE INFLUENCE OF YEAST EXTRACT AND OF PHOTOREACTIVATION

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Growth of the ultraviolet-irradiated cell is of special interest. Growth may offer a simpler measure of radiation effect than survival. Growth after irradiation is a reflection of events in the latent or middle period between the irradiation and the end biological result (Kelner, 1954; Latarjet and Gray, 1954; Kelner et al., 1955; Barner and Cohen, 1956). During this period the ultraviolet damage is partly photoreversible and reactions leading to delayed mutation are preventible (Kelner, 1949; Newcombe, 1956). There may be a relation between abnormal growth processes in the irradiated cell and radiation-induced effects which are characteristically delayed. Since ultraviolet light (2537 A) probably affects the cell nucleus specifically, study of the growth of the irradiated cell may help in solving the problem of the immediate and delayed control of cell growth by the nucleus.

The literature on the effects on microbial growth of ultraviolet light (Kelner, 1953; Errera, 1954; Kanazir and Errera, 1956; Barner and Cohen, 1956; Iverson and Giese, 1957; Deering and Setlow, 1957), and of ionizing radiations (Billen et al., 1953a, b; Latarjet, 1954; Powers and Pomeroy, 1958) may be summarized as follows: cells continue to grow after irradiation at a rate dependent upon dose, but with a dose killing as much as 90 per cent of the cells, the rate is little less than the control. Oxygen consumption is little affected for about the first 90 min, and the synthesis of deoxyribonucleic acid is inhibited more than that of ribonucleic acid, but may resume. And in general, division is more inhibited than growth.

The behavior of cells after ultraviolet light is reviewed by Kimball (1957) and Jagger (1958). Recent research on growth of X-irradiated mammalian cells has been reported by Puck and Marcus (1956). The fundamental studies on growth in Arbacia zygotes (growth considered as division) are extensive (see for example, Blum et al., 1951).

In a previous paper Kelner (1953) showed that Escherichia coli strain B/r incubated in a minimal, defined medium after ultraviolet irradiation in the log phase, continued growing exponentially for a period, after which growth suddenly ceased or became very slow. The present paper describes quantitative experiments on growth after ultraviolet irradiation and photoreactivation, and the effects of nutrition.

MATERIALS AND METHODS

The general techniques were described by Kelner (1953). E. coli strain B/r was maintained on nutrient agar. A "master" M-9 culture was prepared weekly by inoculating M-9 medium² from the slant, and growing the bacteria for two successive subcultures in aerated M-9 at 37 C. The last subculture was diluted with an equal quantity of M-9 salt solution and stored at 5 C. It was used daily for inoculating the culture to be irradiated.

Experiments with M-9 medium. Preparation of log phase cultures for irradiation. For the interrupted growth experiments, M-9 was inoculated from the master M-9 suspension so as to give an initial titer of about 1 X 10⁹ per ml. Tubes (25 by 145 mm) containing 25 to 38 ml of inoculated medium were grown at 37 C with aeration until turbidity measurements showed the bacteria were

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2 Public Health Service Post-Doctoral Research Fellow of the National Cancer Institute.
in the exponential phase and had a titer of from 1 to $6 \times 10^4$ cells per ml. The cultures were pooled and cooled quickly to 20 to 25°C and maintained at this temperature for generally not more than 30 min, while they were irradiated or otherwise manipulated.

For the continuous growth experiments, 80 to 100 ml of M-9 medium inoculated as with the interrupted growth experiments, were dispensed in 140-mm diameter petri dish bottoms. These were partially submerged in a 37°C water bath, and the culture stirred with a magnetic stirrer. Evaporation was retarded with a watch glass.

**Ultraviolet irradiation.** For the interrupted growth experiments, the cooled, pooled suspension was irradiated with 2537 Å from a 15 watt GE germicidal lamp, with an intensity ranging in different experiments from 50 to 90 ergs per mm².

In the continuous growth culture, the suspensions were irradiated without cooling, interrupting aeration, or interfering with growth in any way except by ultraviolet light. The intensity of the ultraviolet light source was 16 ergs per mm².

**Postirradiation growth.** In the interrupted growth method, the irradiated culture was redispensed in tubes and reincubated with aeration at 37°C. The tube was placed in a Beckman model B spectrophotometer for reading of the optical density at wave length 475 mμ, then reincubated at 37°C for further growth. Optical density of the continuous growth cultures was read on 1- to 2-ml samples.

**Photoreactivation.** Experiments on photoreactivation followed those described by Kelner (1953). They were done only with the interrupted growth method. It was difficult to treat continuous growth cultures with a sufficiently high intensity of photoreactivating light, because of the large surface area of the petri dishes containing the cultures.

Protein content of cells was measured by the method of Lowry et al. (1951).

**Experiments with M-9 supplemented with yeast extract.** For the experiments with complex media, stock concentrations of 8 per cent yeast extract (Difco) dried nutrient broth, or casamino acids were prepared in distilled water and autoclaved. The bacteria were grown in M-9, cooled, irradiated, and reincubated at 37°C, still in M-9, for 20 to 30 min. Part of the culture was photoreactivated, and the other incubated in the dark. Then the cultures were diluted 1:5 or 1:6 into fresh M-9 supplemented with stock yeast extract. Unless otherwise noted, the final yeast extract concentration was between 0.55 to 11 mg dry weight per ml of culture. Optical density was measured at 475 mμ, as in the M-9 experiments.

In other experiments the cultures grown and irradiated in M-9 were diluted into yeast-supplemented M-9 immediately after irradiation.

Turbidity in all experiments was expressed in units each of which was equivalent to about 1 × $10^7$ cells per ml (as determined by plate count) of a full-grown M-9 culture, or about 3 × $10^6$ cells per ml of a log phase culture in M-9, with a titer of 1 to 6 × $10^8$ per ml.

Unless otherwise noted all experiments described in this paper were carried out by the interrupted growth method.

**EXPERIMENTAL RESULTS**

**Part 1. Experiments with the synthetic minimal medium, M-9. The basic response; the two phases.** Kelner (1953) reported that postultraviolet exponential growth was followed by a period of complete, or almost complete inhibition of growth; also that the time of the initiation of the period of growth inhibition varied from 60 to 120 min after irradiation.

Reinvestigation of postultraviolet growth with more varied doses and a new strain of *E. coli* (B/r) showed that the first postultraviolet growth period was actually succeeded by a second period in which growth continued, still exponentially, but at a much slower rate. There are then two postultraviolet growth phases, hereafter called “phases 1 and 2,” separated by a sharp break (figure 1). At the doses used previously, and possibly because of strain variation and other unknown reasons, phase 2 was always characterized by sharply inhibited growth, but in the newer experiments at doses still killing all but 1 to 5 × $10^{-2}$ of the cells, growth continued in phase 2.

A third phase of renewed growth, or a “phase 3,” sometimes occurred at about 175 min postultraviolet, especially at low doses, and in yeast-extract supplemented medium. Except where otherwise noted the following discussion will be

4 When total growth measured is low (as in phase 2, or in phase 1 after high doses) it is difficult to distinguish between exponential and arithmetic growth. Since growth before irradiation is exponential, it is simpler to assume without unequivocal contrary evidence, that it remains exponential after irradiation.
limited to growth taking place within the first 150 min only, after irradiation. Phasing occurred in *Escherichia coli* strain B/r growing on M-9 agar.

**Continuous growth experiments.** One step in the interrupted growth method was the cooling and rewarming of cells. Since such temperature changes have been reported to induce synchronous division, the postultraviolet growth phasing was thought to be due perhaps to synchronous increase in turbidity. However, beside the fact that the cooling period in our experiments was not optimal for synchrony, Lark and Maaløe (1956) and others have shown that even where division is synchronous, turbidity increases smoothly.

Experiments, such as that shown in figure 1, in which cells were irradiated during continuous growth without any cooling cycle, show that phasing is not caused by temperature cycling. The chief effect of cooling is to increase the duration of phase 1. The continuous growth experiment shows that the inhibition of growth characteristic of phase 1 is initiated with a lag of less than a minute.

The continuous growth experiments were more uniform than those with interrupted growth, but because of technical inconveniences, were abandoned for the interrupted growth method.

**Protein synthesis during postultraviolet growth.** Dry weight and protein measurement of cells during phases 1 and 2 showed that when the turbidity increased, the protein and dry weight did also. The parallelism between turbidity increase and protein synthesis was not always close, but considering the lesser sensitivity of the direct estimation of protein in small numbers of cells, as compared to the estimation of turbidity (Monod, 1942) it is concluded that the turbidity curves truly represent protoplasmic growth.

**Possible complications due to growth of survivors during phase 2.** When the surviving fraction is 5 × 10⁻² or less, it can be shown that the growth of survivors could not influence markedly the shape of the turbidity curves within the first 150 min after irradiation. They certainly could not account for more than a fraction of phase 2 growth. At low doses when survival is higher, or after 3 hr postirradiation growth, survivor growth may possibly affect the growth curves. The exponential growth in phases 1 and 2 argues against there being two classes of cells in the irradiated population (one of which is more than a negligible fraction of the whole) having different growth rates. Growth of survivors may differ from that of the inactivated cells, but within the first 150 min postultraviolet it can hardly measurably affect
the total optical density, except at the very smallest doses.

Relation of dose to growth inhibition. A family of curves depicting postultraviolet growth of *E. coli* irradiated with a series of doses from 350 to 2800 ergs per mm² is shown in figure 2. Where, as in doses below about 750 ergs per mm² no distinct phase 2 exists, the growth continuing exponentially without a break for at least 150 min, phase 2 is defined as the period, 75 to 150 min postultraviolet.

A critical dose level is about 750 to 1000 ergs corresponding to a survival of about 10 to 20 per cent in strain B/r. Below this, there is no break, occasionally a slight temporary "jog" in the curve. Above 1000 ergs there is increasing inhibition in both phase 1 and 2, phase 2 being more sensitive to the ultraviolet light. At doses of about 3000 ergs per mm² and higher, postirradiation growth is almost completely inhibited in both phases.

Figure 3 shows the curve for the relative growth rate in phases 1 and 2 versus dose. The relative growth rate of irradiated cells is taken to be the time for doubling of turbidity of nonirradiated controls divided by the doubling time for irradiated cells. The curve for phase 1 is exponential up until at least 3000 ergs and differs distinctly from the sigmoid curve for phase 2, which resembles the dose-survival curve for *E. coli* B/r.
The break. Many experiments, in which optical density readings were taken a minute or two apart in the region of the break, established that the transition in growth rate occurred within 5 min or less. This was especially clear for ultraviolet doses (1500 to 2500 ergs) where the growth rates of phase 1 and 2 differed markedly. Once this fact had been established, readings so close together were no longer made routinely, and the sharpness of the break was assumed.

Strains of *E. coli* varied in break time from 75 to 100 min (interrupted growth experiments). For the strain used in most of these experiments, the break time was uniformly 70 to 75 min in interrupted growth experiments, and 60 to 65 min in continuous growth experiments.

The break time did not vary with dose (table 1), except for an occasional slight lengthening at the highest doses.

*Extrapolation to 2.* The only experimental variable found to affect break time was the temperature of postultraviolet incubation. In the range 28 to 37 C, the lower the temperature the more prolonged the break time. For example, at 31 C, in a continuous growth experiment the break time was 98 min. At temperatures below 37 C, the sharpness of the break was even more pronounced than at 37, due perhaps to a greater relative inhibition of growth in phase 2 as compared to phase 1.

There was no simple, consistent, and temperature-independent relation between the generation time of nonirradiated cells, and the break time of irradiated cells. The cells obviously did not have to reach a critical mass before shifting growth rate, since even with severely inhibited growth, the break time was about the same as at a lesser dose.

A possibly significant relationship was the log of the relative increase in mass (turbidity at the time of the break divided by the turbidity immediately after irradiation) to ultraviolet dose. Figure 4 shows a curve of the log of the increase in mass versus ultraviolet dose for a continuous and an interrupted growth experiment at 37 C. The curve is a straight line extrapolating at zero dose to two.

**Figure 4.** Log of relative increase in mass during phase 1 versus ultraviolet (UV) dose in ergs per mm². Data from continuous growth experiments in M-9 at 37°C, and the interrupted growth experiment shown in figure 2. For comparison, points are also shown of continuous growth experiments at 31 to 33°C. The relative increase in mass is estimated by dividing the turbidity units at the time of the shift in growth rate, or the break, by the turbidity units immediately after irradiation. The curve extrapolates to two at zero dose. The difference in slope of the continuous and interrupted growth experiments is not considered significant.

<table>
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<th>Dose, in Ergs per mm²</th>
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<td>2900</td>
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**Table 1.** Dose-independence of the duration of phase 1 (break time). Continuous growth experiments.
growth of the more sensitive strain B was scarcely inhibited, nor was there clear-cut phasing. Thus, when survival was the criterion for dose the radiation-resistant strain B/r was apparently more sensitive than strain B. When, however, the two strains were given the same absolute dose, regardless of the difference in survival caused by this dose, strain B showed typical growth inhibition, a break, and phasing. In fact, the curves for the two strains were almost superimposable (figure 5). It was clear that when strain B was irradiated with a dose giving about 5 $\times$ 10$^{-3}$ survival, it was being irradiated with a low dose so far as growth inhibition was concerned, and the lack of growth inhibition by ultraviolet light was what would be expected if the ultraviolet sensitivity of strain B for the lethal and for the growth inhibiting action of ultraviolet light was different.

Five other strains of B and B/r cultures, including a tryptophan-requiring strain B/r, from 5 different laboratories, differed only in minor ways from our own strains B and B/r. The strains of B and B/r showed typical photoreactivation of ultraviolet-induced growth inhibition.

E. coli strain K-12s and K-12r showed typical postultraviolet behavior; in strain K-12s, two exponential phases; in strain K-12r, a phase 1 followed by lysis. The growth patterns were quite distinct, indicating that the postultraviolet phasing described in the present paper was not correlated with the presence of a temperate phage.

**Effect of photoreactivating light.** Growth of non-irradiated controls treated with photoreactivating light was inhibited about 10 per cent. The effect of photoreactivating light on phase 1 of irradiated cells is difficult to measure, because an appreciable portion of phase 1 is taken up by the 20 to 30 min exposure to reactivating light itself. Although light does stimulate growth in phase 1, its effect is most marked for phase 2 and the discussion will be confined to this phase. With ultraviolet doses, about 500 to 1250 ergs per mm$^2$, reactivating light restores growth to a level almost to that of the control abolishing phase 2, whereas at higher doses (ca. 2000 ergs) there is a phase 2, with the break occurring at about the same time as in the nonphotoreactivating cells (figures 6–8).

Phase 2 in reactivated cultures is exponential and usually remains so for at least 4 hr. The data are consistent for a dose reduction (Kelner, 1949), caused by light, of about 2 (figure 6). The rate of protein synthesis in reactivated cultures is restored to about the same degree as is the rate of turbidity increase.

**Loss in photoreactivability.** An attempt was made to correlate postirradiation growth phasing with the loss in photoreactivability known to occur if irradiated resting cells were incubated in the dark at 37 C before exposure to reactivating light (Kelner, 1949). It was thought that the sudden shift in growth rate at the break might be paralleled by a sudden and pronounced loss in photoreactivability.

Cells in the exponential phase were irradiated and incubated in the dark at 37 C, and after various intervals were tested for photoreactivability. The ability of light to stimulate growth or to increase the number of survivors were both
lost gradually as the period of incubation before reactivation was increased, reaching zero at about 100 min after irradiation.

Growth of survivors after irradiation. The discussion so far has dealt chiefly with postirradiation growth of inactivated cells. An important question was whether the growth of the potential survivors, existing immediately after ultraviolet, also is inhibited and characterized by two phases.

Examination of survivor growth is far more difficult than turbidimetric measurement of the total mass of cells, where survivors and nonsurvivors are not differentiated. The usual plate counts measure numbers of cells irrespective of their size, whereas turbidimetry measures total mass, irrespective of number. Colony counts of irradiated cells are also complicated by recovery phenomena.

The term, survivor, denotes the potential ability to divide indefinitely. Although the decisive ultraviolet-induced change determining future behavior may be already present in each cell immediately after ultraviolet irradiation, the change may not yet have caused any behavior differences. Environmental factors occurring hours later may finally bring the cell to the nonreactive state equaling death.

Some idea of the relative growth of survivors may be obtained by studying growth curves after low doses. The curves for 350 and 700 ergs in figure 2 are exponential for at least 200 min. Survival at these doses with the strain used was 65 and 35 per cent, respectively. If survivors were growing faster than the inactivated cells, the turbidity curve would bend upward by 100 to 150 min after irradiation. Also, the curve would not remain exponential if the survivors were growing at a rate much less than that of the inactivated cells. Unless some complex combination of a multicomponent population with varying growth rates and lags, interacting to give an over-all exponential growth, is hypothesized, a reasonable conclusion is that at about 500 ergs per mm² survivors and nonsurvivors both grow at the same inhibited rate.

Survivor growth in M-9 after irradiation was followed by plate counts. At doses between 1050 and 2100 ergs growth, i.e., increase in numbers of survivors continued usually without a lag and at a rate higher than that shown by the turbidity curves for inactivated cells. But in cultures which were irradiated with 2100 ergs, then photoreactivatated, survivor numbers and over-all turbidity both increased at nearly the same rate.

Part 2. Effect of yeast extract on postultraviolet growth. The effect of yeast extract on postirradiation growth was studied because the ability of cells to respond to nutrient changes by changes in growth was a fundamental characteristic of the integrated normal cell. It was of interest to see how a cell with an injured nucleus would respond to complex nutrients. Also, yeast extract has been reported to increase survival of X-irradiated *Escherichia coli* (Stapleton et al., 1955), but not in *Serratia indic a* (Davydoff, 1956) or spores of *Bacillus subtilis* (Donnellan and Morowitz, 1957). In addition, the presence of complex nutrients postultraviolet was found to influence mutagenesis (Witkin, 1956; Haas and Doudney, 1957).

Nonirradiated cells grown to log phase in M-9, then diluted into M-9 supplemented with 11 mg per ml of yeast extract, responded to the supplement by an immediate acceleration in growth, with no lag (figure 7). The doubling time in M-9 was 45 min; in yeast extract supplemented M-9, 27 min.

Analysis of the action of yeast extract on growth of irradiated cells was complicated by an unexpectedly high loss in optical density when
The exact amount of loss varied from experiment to experiment. Dilution of a comparable irradiated culture into fresh M-9 resulted in precisely the expected turbidity.

The loss in optical density proved to be dependent within limits upon a postirradiation incubation period in M-9 before dilution; was proportional to the concentration of yeast extract; was produced also by nutrient broth or mixtures of amino acids; was dependent upon the ultraviolet dose, being absent at doses under about 1200 ergs per mm²; and was absent in nonirradiated, or in photoreactivated cells.

Although the loss in optical density did not occur if cells were diluted immediately into yeast extract medium after irradiation, nevertheless whatever caused the phenomenon might still be operating during postirradiation growth in yeast extract and cause a spuriously low rate of growth.

Because of this complication, a precise description of the effect of yeast extract on growth during phase 1 was impossible. It was clear, however, that in contrast to its effect on nonirradiated cells, yeast extract stimulated growth very little, if at all, during the first 75 min or so after irradiation (figure 7).

Because the effect of yeast extract on phase 2 was more regular than on phase 1, most of our discussion will be on phase 2. At doses of about 2500 ergs per mm², irradiated cells grown in yeast extract medium shifted in growth rate at about the same time as those grown in M-9 (figure 7). At intermediate doses (about 1500 ergs), the combination of depressed growth in phase 1, with relatively little inhibition of growth by the low dose of ultraviolet in phase 2, often gave curves not showing phasing.

At doses of about 1500 to 2500 ergs, phase 2 (75 to 150 min postultraviolet period) was exponential in yeast extract medium and the growth rate was greater than in M-9 (table 2 and figure 7). The ratio of growth rates (doubling time in M-9 divided by doubling time in yeast extract medium) for phase 2 averaged 1.94, compared to the ratio for nonirradiated controls of 1.63 (table 2). Although individual experiments were variable, the conclusion is that during phase 2 yeast extract stimulates growth of ultraviolet-irradiated cells about to the same degree proportionately as it does the control cells, with the relative stimulation tending to be greater with increasing ultraviolet dose (table 2).
In summary, yeast extract clearly stimulates phase 2 growth, inhibiting or stimulating growth very little in phase 1, and does not affect break time. The response to yeast is complex and complicated by effects on optical density.

A curve expressing the relation of ultraviolet dose to the relative inhibition of growth during phase 2 in yeast extract medium resembles that shown in figure 3 for phase 2 in M-9. *Photoreactivating light.* Reactivating light inhibited nonirradiated cells growing in yeast extract medium by about 10 per cent, just as it did cells growing in M-9.

Photoreactivated cells responded to yeast extract by an immediate acceleration in growth just as did nonirradiated cells, with no lag (figures 2-13). Phasing in photoreactivated cells occurred only with doses of about 2500 ergs or higher (figure 7). Reactivated cells rarely exhibited a phase 3 up to 4 hr after irradiation.

The ratio of the doubling time in phase 2 of reactivated cells in M-9 to those in yeast extract medium averaged 1.8, similar to that of nonirradiated controls (table 2). The ratio was independent of dose within the range 1500 to 2500 ergs, except for an irregular tendency to increase at the highest doses.

**Loss in photoreactivability.** As in the comparable experiments in M-9, continued postultraviolet incubation in the dark, in yeast extract medium, resulted in a progressive loss of the ability of light to stimulate growth. At 80 to 100 min, postultraviolet light had little effect on growth. Loss in photoreactivability was shown both by a reduction in the acceleration in growth due to light, and by increased lag between the application of light, and the stimulation in growth.

Photoreactivation, then, restores the ability of irradiated cells to respond to yeast immediately and without a lag, prevents the loss in optical density on dilution into yeast extract medium, and usually prevents a phase 3. The fact that there is an exponential curve in reactivated cells grown in yeast extract medium at doses under 2500 ergs, for about 250 min postultraviolet, indicates that in reactivated cultures, the potential survivors grow at a rate similar to the nonsurvivors. Exposure of cells to light before irradiation had no effect on subsequent inhibition of growth by ultraviolet light.

### TABLE 2

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<td>1.81</td>
<td>1.94</td>
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All growth rates determined during phase 2, or 75 to 150 min postirradiation.

* CM/CY: doubling time in nonirradiated cells grown in M-9 divided by doubling time for those grown in yeast extract medium. LM/LY: doubling time of photoreactivated cells grown after irradiation in M-9 divided by doubling time cells similarly treated but grown after irradiation in yeast extract medium. DM/DY: As LM/LY, but not photoreactivated. All cells grown in M-9 before ultraviolet irradiation or yeast extract supplementation.

† Not included in the average.
TABLE 3

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Dose (Ergs per mm²)</th>
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</table>

* Doubling time (minutes) in nonirradiated controls divided by doubling time in photoreactivated cells.

Relation of dose to growth inhibition. Table 3 summarizes 10 experiments in which the effect of dose on the growth rate of reactivated cells in yeast extract medium and in M-9 is compared with that of nonirradiated controls. The postirradiation period considered was 75 to 150 min (phase 2). The ratios obtained for the same dose differed from experiment to experiment. For example, at 1980 ergs the ratio of doubling time in nonirradiated controls to that in reactivated cells in yeast extract medium varied from 0.47 to 0.77. This variability may have been due to different levels of photoreactivation in different experiments. A plot of the data in table 3 (ultraviolet dose versus growth inhibition in photoreactivated cells) gives a curve clearly different from the exponential curve in figure 3 for phase 1 inhibition of nonphotoreactivated cells, and resembles more the sigmoid phase 2 curve in figure 3 with a dose reduction of about two.

The assay for the effect of reactivating light on growth of irradiated cells is quantitatively greater if yeast extract medium is used, although the actual degree of stimulation may be intrinsically no greater than in minimal medium.

Effect of supplements other than yeast extract. Various nutrients were compared with yeast extract on a weight for weight basis for stimulation of growth of photoreactivated cells. Figure 8 shows that nutrient broth (4 mg per ml), yeast extract, and amino acid mixtures (both 10 mg per ml) stimulate equally. In other experiments in which the concentrations of the supplements were varied, this general similarity was confirmed. At the level of 10 mg per ml, nutrient broth was, however, somewhat more effective than yeast. Glucose at 10 mg per ml added to M-9, was if anything slightly inhibitory. The same general effect of the various supplements was noted on nonreactivated cells, although the assay was less exact than in photoreactivated cells. Only yeast extract was tested at trace levels.

Stapleton and co-workers (1955) had reported that glutamine, uracil, and guanine, plus salts, resembled yeast extract in causing recovery in colony-forming ability under certain conditions in X-irradiated E. coli. The possibility that these supplements were the active components of yeast extract in stimulating growth was therefore tested. They did not stimulate growth in our experiments. The addition of ammonium tartrate or NH₄Cl to the supplements, as recommended by the authors for increasing colony size of the recovered cells, also failed to stimulate growth of irradiated cells grown with or without photoreactivation.

Nutrition before irradiation. Stapleton and co-workers (1955) found that yeast extract induced
recovery only in cells which had been grown in yeast extract or nutrient broth before X-irradiation. We thought that the negative effect of glutamine, uracil, and guanine in our own experiments might be because we used cells grown in M-9.

Bacteria grown overnight in M-9 were transferred to nutrient broth and incubated until they reached log phase (2 to 3 hr), then washed in M-9, irradiated, and subsequently treated as in a standard experiment: grown 25 minutes in M-9 with and without reactivating light, then diluted into M-9, M-9 plus glutamine, uracil, and guanine; or plus yeast extract or nutrient broth.

Nonirradiated broth-grown controls behaved very much like M-9-grown cells. Irradiated broth-grown cells also resembled M-9-grown ones in their response to yeast extract, nutrient broth, a mixture of guanine, uracil and glutamine, and reactivating light. The growth inhibitory action of ultraviolet light was possibly greater for broth-grown than for M-9-grown cells.

**Effect of yeast concentrations.** Concentrations of yeast extract of 0.03 to 20 mg per ml were tested for ability to stimulate growth of photoreactivated cells. Concentrations from about 0.66 to 20 mg per ml stimulated growth equally. The cells responded immediately and maximally. With concentrations of 0.03 to 0.66 mg per ml, the cells responded immediately and at a maximal rate, then after a period, proportional to the yeast concentration, reverted sharply to the M-9 rate (figure 9).

A similar qualitative effect was noted for trace yeast concentrations in nonirradiated cells. Non-photoreactivated cells were not tested because of the complex response of such cells to yeast extract, and their over-all low rate of growth.

**Phase 3.** In M-9, exponential growth of phase 2 continued up to 200 min after irradiation. In yeast extract medium, phase 2 was often, but irregularly, followed by an acceleration in growth starting about 150 to 175 min after irradiation. Phase 3 differed from phase 2 in several ways: it occurred most often with doses below 2000 ergs per mm²; the greater the dose the later phase 3 started; its inception was not sharp. Phase 3 usually terminated in an exponential growth rate which continued unchanged for the duration of the longest experiment, 500 min post ultraviolet. This terminal growth rate was slower the greater the ultraviolet dose and never equalled the rate of nonirradiated controls. Phase 3 was rarely found in photoreactivated cultures.

![Figure 9](http://jb.asm.org/ Downloaded from http://jb.asm.org on November 13, 2017 by guest)
Table 4

Estimated contribution of survivor growth to growth during phase 3 in irradiated cells grown postultraviolet in yeast extract medium

<table>
<thead>
<tr>
<th>Minutes Post-irradiation</th>
<th>Survivors (Plate Count)</th>
<th>Estimated Turbidity Contributed by Survivors* (Turbidity Units)</th>
<th>Over-all Turbidity of Culture (Turbidity Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$1.5 \times 10^6$</td>
<td>1.8</td>
<td>20.7</td>
</tr>
<tr>
<td>35</td>
<td>$3.0 \times 10^6$</td>
<td>3.5</td>
<td>24.9</td>
</tr>
<tr>
<td>70</td>
<td>$1.7 \times 10^6$</td>
<td>2.0</td>
<td>26.6</td>
</tr>
<tr>
<td>90</td>
<td>$3.2 \times 10^6$</td>
<td>3.8</td>
<td>29.3</td>
</tr>
<tr>
<td>135</td>
<td>$1.0 \times 10^7$</td>
<td>12.0</td>
<td>39.2</td>
</tr>
<tr>
<td>180</td>
<td>$4.3 \times 10^7$</td>
<td>51.0</td>
<td>72.5</td>
</tr>
</tbody>
</table>

Plate count on M-9 agar. Dose, 1950 ergs per mm². A pronounced phase 3 starts at about 135 min.

* Turbidity contributed by survivors estimated by multiplying plate count by the factor, 1 turbidity unit per $8.5 \times 10^6$ cells.

(Many experiments had shown that survivor assay in M-9 and nutrient agar were similar.) A pronounced phase 3 started about 150 min postirradiation. The contribution of the survivors to the total turbidity of the cultures was estimated by multiplying the plate count by the factor $1/(8.5 \times 10^6)$, a factor obtained by dividing the turbidity of nonirradiated control cells grown in yeast extract medium by the plate count. For comparable M-9 grown cells the factor was $1/(3 \times 10^6)$, a difference explainable by the larger size of cells grown in yeast extract medium.

Table 4 shows the survivor number, over-all turbidity, and estimated contribution of the survivors to the over-all turbidity. At 135 min post-ultraviolet, approximately 30 per cent of the total turbidity could be ascribed to the survivors; at 180 min, 70 per cent, more than enough to account for phase 3, although other hypotheses cannot be excluded.

The terminal growth rates in phase 3 can be explained by survivors having a growth rate during the period of study less than that of the control, and equal to that of phase 3, and being dose dependent; the delay in phase 3 by the increase in dose causing a lower proportion of survivors; the absence of a phase 3 in photoreactivated cells by a similarity in growth rates of survivors and nonsurvivors in such cells.

Discussion

Ultraviolet-induced effects on growth should be added to other ultraviolet effects. In which strains B and B/r respond similarly to absolute dose: mutagenesis (Demerec and Latarjet, 1946) and inhibition of adaptive enzyme synthesis (Kaplan et al., 1953). The great sensitivity of strain B to the lethal action of ultraviolet may not be closely related to the other effects.

The loss in optical density of ultraviolet irradiated E. coli caused by yeast extract is apparently the result of some alteration in the membrane properties of the cell by ultraviolet light. It resembles similarly rapid losses in optical density followed by a recovery on changes in menstruum tonicity reported for gram-negative bacteria, including E. coli strains B and B/r (Avi-Dor et al., 1950).

Some reasons that postultraviolet phasing has been so rarely reported have perhaps been that the dose used by many workers has been too low; resting cells were irradiated; or the growth in the first 100 min after irradiation was not followed closely. Rubin (1954) published graphs which may indicate phasing in E. coli exposed to ionizing radiation. Biphase posttreatment growth in Saccharomyces cerevisiae exposed to nitrogen mustard was found by Hutchens and Podolsky (1954), and in the flagellate Strigomonas oncopelti treated with ethidium bromide, by Newton (1957). In Newton's study, however, increase in cell numbers rather than cell mass was the criterion for growth.

Shifts in growth rate due to exhaustion of nutrilites in the medium have been described by Monod (1942). Indeed, the temporary acceleration of growth with small concentrations of yeast in our own experiments is a good example of biphase growth due to external factors (figure 9).

There is also a resemblance between postultraviolet growth phasing and the growth pattern in enucleated Acetabularia (Brachet and Chantrenne, 1956).

The two phases in postultraviolet growth are probably the result of events within the cell and not to some external factor, such as the exhaustion of some nutrient. This is shown by the break time remaining constant over a wide range of ultraviolet doses, when growth (which would be expected to exhaust substances in the medium)
varies from almost no increase in turbidity to an increase of about 170 per cent. Also break time is little affected by concentration of cells or by dilution after irradiation into fresh medium.

A clue to what is happening may be found in the fact that the relative increase in mass during phase 1 versus dose extrapolates to two at zero dose (figure 4). A tentative theory supposes that a cellular component doubles every 45 min in the normal cell growing in M-9. Growth is synchronized with the doubling of this component, therefore the mass of the normal cell doubles every 45 min. A very low dose of ultraviolet switches the doubling time of this component to 65 min, thereby changing the doubling time for growth to 65 min. With an increasing dose, the doubling time of the component remains constant at 65 min; growth is no longer synchronized with it, but is inhibited proportionately to the dose. The duration of phase 1 depends upon the doubling time of the component (65 min). If the component is the nucleus or a part of it, then break time can be related to some part of the division mechanism of the cell.

Growth inhibition in phase 1 (exponential curve, figure 3) might be explained on the assumption that the growth rate in a normal cell is proportional to the concentration of a large number of growth-controlling molecules or particles, which are inactivated by ultraviolet light on a 1-hit basis.

Whatever the theoretical basis of the observations, the evidence is clear that at least two pacemakers are concerned in postultraviolet irradiation growth. One is probably influenced by the availability of amino acids or other factors in yeast extract, and is independent of dose, whereas the other is profoundly influenced by ultraviolet light, may be associated in part with the nucleus, and is sensitive to photoreactivating light.

**SUMMARY**

Log phase cultures of *Escherichia coli* strain B/r, grown in minimal medium, were exposed to a series of ultraviolet doses and growth following irradiation was evaluated turbidimetrically. Post-irradiation growth was characteristically biphasic.

Phase 1, occurring immediately after irradiation, and without a lag, had an exponential and an inhibited growth rate which was dose-dependent. The ultraviolet dose versus growth inhibition curve was exponential.

With continued postirradiation incubation, the growth rate of phase 1 shifted suddenly to the slower but still exponential growth rate of phase 2. The time of the shift was independent of dose or the amount of growth that had preceded it.

The cause of the shift in growth rate was unclear. The shift is possibly triggered by the doubling of some cellular component during phase 1, a theory suggested by the fact that the curve for the relative increase in cell mass during phase 1 versus ultraviolet dose, extrapolated to two at zero dose.

The growth rate in phase 2 was also dose-dependent; the ultraviolet dose versus growth inhibition curve was sigmoid and resembled the dose-survivor curve for *E. coli* strain B/r.

The growth inhibitions were photoreversible, with a dose reduction of about 2, for phase 2.

Yeast extract added to the minimal medium did not influence markedly the characteristically biphasic growth after ultraviolet irradiation. It stimulated growth of irradiated cells very little if at all during phase 1. During phase 2 it stimulated growth of irradiated, photoreactivated, and control cells about to the same proportion, showing that the growth stimulating action of reactivating light and yeast extract were independent.

Nutrient broth and a mixture of amino acids produced the same effect of yeast extract, but a mixture of guanine, uracil, and glutamine, were ineffective.

If *E. coli* strains B and B/r were irradiated with a dose giving the same survival, strain B was much less inhibited in growth; but if irradiated with the same absolute dose, their postultraviolet growth was similar.

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