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Changes in the Ultraviolet Sensitivity of *Escherichia coli* During Growth in Batch Cultures

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The ultraviolet (UV) sensitivity of *Escherichia coli* B/r harvested at various times during growth in batch cultures was measured. The results showed a period of increased UV sensitivity in late log phase, just before the cultures entered stationary phase. This increase in sensitivity was associated with a decreased shoulder in the UV survival curves. The postirradiation division delay of survivors was shortest for cells harvested during the period of maximal sensitivity. This period of increased UV sensitivity during late log phase was not found in the radiation-sensitive, repair-deficient mutant B_{s-1} (a strain which is unable to excise pyrimidine dimers from UV-damaged deoxyribonucleic acid). These results suggest that the variation in UV sensitivity of *E. coli* B/r as a function of time of harvesting of the cells from batch cultures is related to the varying capacities of these populations to repair UV-damaged deoxyribonucleic acid. Further experiments designed to elucidate the mechanism underlying this variation in UV sensitivity indicated that it arises from the partial depletion of nutrients in the medium during late log phase. We suggest that growth in such depleted media leads to a depression in the intercellular concentration or activity of one or more of the repair enzymes concerned with the repair of damaged deoxyribonucleic acid.

Batch culture is the most commonly used method of growing bacterial cells, but, as has long been known, many of the properties of such cells depend on the stage of growth at the time of harvesting (18). An example relevant to this work is the fluctuation in the X-ray sensitivity of *Escherichia coli* B/r that occurs during the log phase (1, 15). In principle, this and other related phenomena could be attributed to two, not mutually exclusive, causes: (i) changes in the magnitude of some cellular property (it being assumed for practical purposes that all cells are identical), and (ii) changes in the distribution of this property among individual cells of the population. The latter might arise because of changes in the age distribution of the population owing to the increase in cell division time that occurs near the end of log-phase growth (in unirradiated populations). Variations in enzyme activity during culture growth are probably an example of the first type of process, and changes in the average number of "nuclei" per cell would be an example of the second.

Recent studies have shown that the sensitivity of bacteria to a wide variety of inactivating and mutagenic agents is dependent upon the activity of various "repair enzymes" (9, 11). The enzymes concerned with the excision of pyrimidine dimers from ultraviolet (UV) damaged deoxyribonucleic acid (DNA; 4) and those capable of rejoining broken DNA strands (6, 17) are examples of such repair enzymes. In this paper, we will describe the changes during batch-culture growth in the UV sensitivity of two *E. coli* strains, a resistant one that repairs UV damage (*E. coli* B/r), and a sensitive one, *E. coli* B_{s-1}, that lacks the ability to excise pyrimidine dimers from DNA (14, 16). The pattern of these changes is surprisingly similar to changes in the luminescence emitted by growing cultures of *Photobacterium fischeri*, recently reported by Kempner and Hanson (13). Although this similarity may be only coincidental, it is worth pointing out that our interpretation of the fluctuation in UV sensitivity differs in a very fundamental way from the explanation of the luminescence changes given by Kempner and Hanson.

MATERIALS AND METHODS

Culture techniques. The *E. coli* B/r used in these experiments was ATCC 11303; our sample of *E. coli* B_{s-1} was provided by Ruth F. Hill. The cells were maintained on Nutrient Broth (Difco) slants (0.8%) containing 0.5% NaCl and 1.5% agar. The same

Nutrient Broth medium was also used without agar. The minimal medium (either with or without 1.5% agar) contained (per liter of distilled water): citric acid, 1.0 g; Na_2HPO_4, 4.6 g; NH_4Cl, 0.45 g; K_2HPO_4, 0.70 g; KCl, 0.10 g; and $MgSO_4 \cdot 7H_2O$, 0.09 g. Sterile glucose was added after autoclaving. A concentration of 0.05% limited growth to about 10^9 cells/ml. The growth temperature was always 37 C. Broth cultures were incubated on a reciprocating shaker to provide aeration. Viable cells or survivors were determined by plating on nutrient agar medium (overnight incubation) or on minimal medium (2 days of incubation). The total cell titer, which agreed within experimental error with the viable cell count, was determined by use of a model B Coulter counter.

UV survival experiments. UV survival experiments were begun by inoculating medium with cells from a culture grown overnight on the same medium. To assay for the UV sensitivity, cells were first harvested, resuspended in buffer (the minimal medium without glucose), and exposed in a thin layer, essentially transparent, to UV. The UV source was a 15-w General Electric germicidal lamp. The dose rate was measured with an Epply thermopile, calibrated against a National Bureau of Standards lamp, and the component transmitted by Pyrex was subtracted from the total. For experiments with *E. coli* B/r, the dose was 12 ergs per mm^2 per sec, and for *E coli* B_{s-1} it was 0.45 ergs per mm^2 per sec.

RESULTS

The UV sensitivity of *E. coli* B/r cells harvested at different times from a Nutrient Broth culture is shown in Fig. 1. The cells were exposed to a constant dose of 950 ergs/mm^2 and were plated on either Nutrient Broth agar or minimal agar. These results show that a period of increased UV sensitivity develops in mid-log phase and reaches its maximum just before the cells enter the stationary phase; resistance returns during the stationary phase. This same pattern has been observed for cells grown in several different kinds of medium, including minimal glucose-salts medium, though the quantitative details vary somewhat among the different media.

The fluctuation in sensitivity shown in Fig. 1 arises primarily from changes in the shoulder of the UV survival curve, as shown in Fig. 2. During the sensitive period, the shoulder was reduced though the curves remained essentially parallel at higher doses. We should emphasize that this result was obtained for cells plated on Nutrient Broth agar. As Fig. 1 shows, there was clearly a difference in response to UV (not apparent in Fig. 2) between early log-phase cells and stationary-phase cells. This difference was revealed by plating on minimal medium. During early log phase, when there was a difference between survival on complete and minimal medium, the survival curves obtained by plating on minimal medium

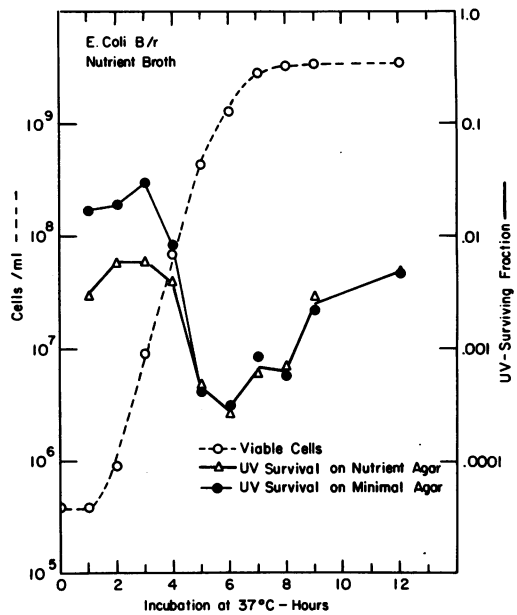

FIG. 1. *UV sensitivity of E. coli B/r during growth in Nutrient Broth medium. Cells were washed, resuspended in buffer, and irradiated with 960 ergs/mm^2. Identical portions were plated on minimal agar* (○, ●) *and on Nutrient Agar* (△, ▲).

were more complex than those shown in Fig. 2. Alper and Gillies (2, 3) have discussed the effects of plating medium on the survival of *E. coli* B and B/r.

These results indicate that there are at least three distinct phases in the cycle of UV sensitivity of *E. coli* B/r that may be distinguished as follows: "early log phase" (plating medium effect), "late log phase" (increased sensitivity), and stationary phase. The lag phase probably constitutes a fourth phase, but was not included in this study.

The changes in UV sensitivity shown in Fig. 1 and 2 are also correlated with postirradiation division delay. We measured the growth of UV-irradiated *E. coli* B/r in Nutrient Broth by plating at intervals on Nutrient Broth agar to determine the number of viable cells present. The logarithmic portion of each growth curve was extrapolated back to the abscissa, and this time intercept used as a measure of "division delay." The procedure was repeated for cells harvested from each of the three stages of growth indicated in Fig. 2. The results (Fig. 3) show that postirradiation division delay at essentially all UV doses was least for cells harvested in late log phase, at the time of maximal UV sensitivity.

The above results suggest that postirradiation processes are somehow involved in the observed

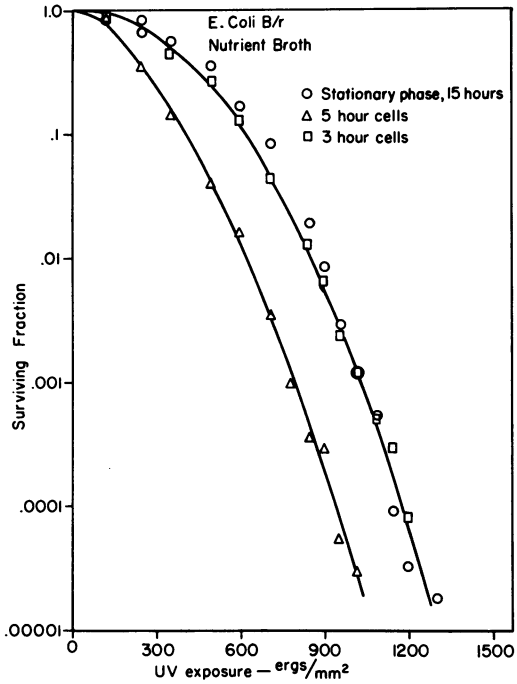

FIG. 2. *UV survival curves at three different times during the growth of an E. coli B/r culture in Nutrient Broth medium: (○) during stationary phase after 15 hr of growth; (△) log phase after 5 hr of growth, giving a titer of 5 × 10⁸ cells/ml; (□) log phase after 3 hr of growth, giving a titer of 10⁸ cells/ml. The growth curve for these cells is essentially the same as that shown in Fig. 1.*

fluctuations in UV sensitivity. Thus, it would appear that the ability to repair UV damage may depend on the time at which cells are harvested from batch cultures. To make a more significant test of this, we repeated the experiment of Fig. 1 for the repair-deficient strain B_{s-1}. The results are shown in Fig. 4 for two different UV doses, 4.5 and 9 ergs/mm². The pattern is quite different from that obtained with *E. coli* B/r; no similar period of increased UV sensitivity occurred during culture growth.

We have carried out several experiments designed to explore the relationship between the increase in UV sensitivity and exhaustion of the medium. In the first, we compared growth and UV sensitivity of *E. coli* B/r using fresh Nutrient Broth, "conditioned" Nutrient Broth, and "reconstituted, conditioned" Nutrient Broth. The "conditioned" medium was prepared by growing a culture to 5 × 10⁸ cells/ml, centrifuging to remove cells, and sterilizing by membrane filtration (Millipore Corp., Bedford, Mass.). The "reconstituted, conditioned" medium was the

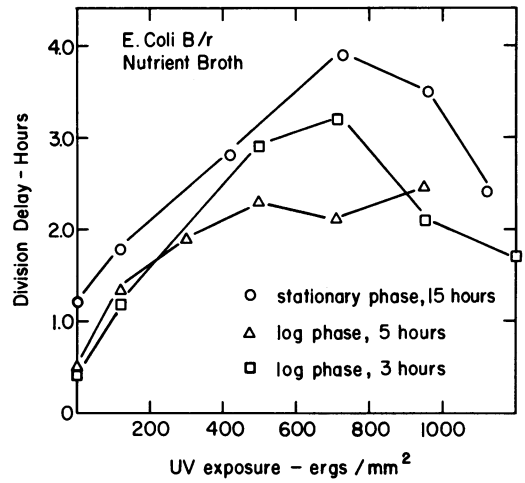

FIG. 3. *Division delay following UV irradiation of E. coli B/r. The cells were grown in Nutrient Broth medium to the same titers as in Fig. 2 and then irradiated; subsequent growth of viable cells in Nutrient Broth was measured by plating on Nutrient Broth agar. The division delay was calculated as described in the text. The UV survival is the same as that given in Fig. 2. Three separate experiments, one for each part of the growth cycle, are shown.*

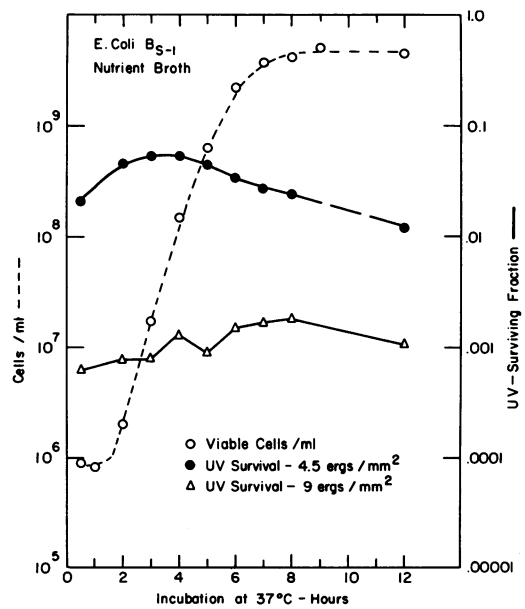

FIG. 4. *UV survival of E. coli B_{s-1} after a constant dose as a function of the growth phase of the bacterial culture. Growth in viable cells per milliliter in Nutrient Broth medium is shown by the dashed line; UV survival is shown by solid lines. The two doses used were 4.5 ergs/mm² (●) and 9 ergs/mm² (△).*

same, except that before sterilizing we added solid Nutrient Broth and NaCl to the usual concentrations. The *p*H of the fresh and "conditioned" media were the same, 6.6; that of the "reconstituted, conditioned" medium was slightly more acid, 6.4. To compare these media, we chose to approximate balanced growth conditions. Initially a culture was batch-grown to 10^7 cells/ml. After dividing the culture into three portions, we diluted it every 10 min with additional medium, the amount being chosen to maintain the cell titer between 0.8×10^7 and 1.5×10^7 cells/ml. Every 20 min, the cultures were divided so that the total volume would not become unmanageable. As shown in Fig. 5, when the growing culture was diluted with fresh medium the doubling time was about 20 min, and after about nine generations the UV sensitivity was still essentially the same as that of cells batch-grown to 10^7 cells/ml. That is, the UV sensitivity did not change. If, however, the culture was diluted with "conditioned" medium, the growth rate was significantly depressed (average doubling time was about 23 min). It should be remembered that the original medium was only gradually replaced with "conditioned" medium. At the end of about eight generations of growth, the UV sensitivity was greatly increased, and was about the same as for cells batch-grown to 5×10^8 cells/ml (the titer to which the cells had been grown to produce the "conditioned" medium). Finally, Fig. 5 shows that the addition of fresh nutrients to the "conditioned" medium restored the growth rate to normal and changed the UV survival curve to that of the control. The results of this experiment indicate either that there is some factor or inhibitor in "conditioned" medium which causes increased UV sensitivity (and which is counteracted by fresh nutrients) or that there is a factor in fresh nutrients which causes increased UV resistance (but which is absent in "conditioned" medium).

The second experiment, shown in Fig. 6 and 7, distinguishes between these two alternatives. Cells grown in minimal medium (and plated on Nutrient Broth agar to assay survival) had a period of increased UV sensitivity, as shown in Fig. 6 (the constant UV dose in this case was 960 ergs/mm^2). The first indication of an increase in sensitivity

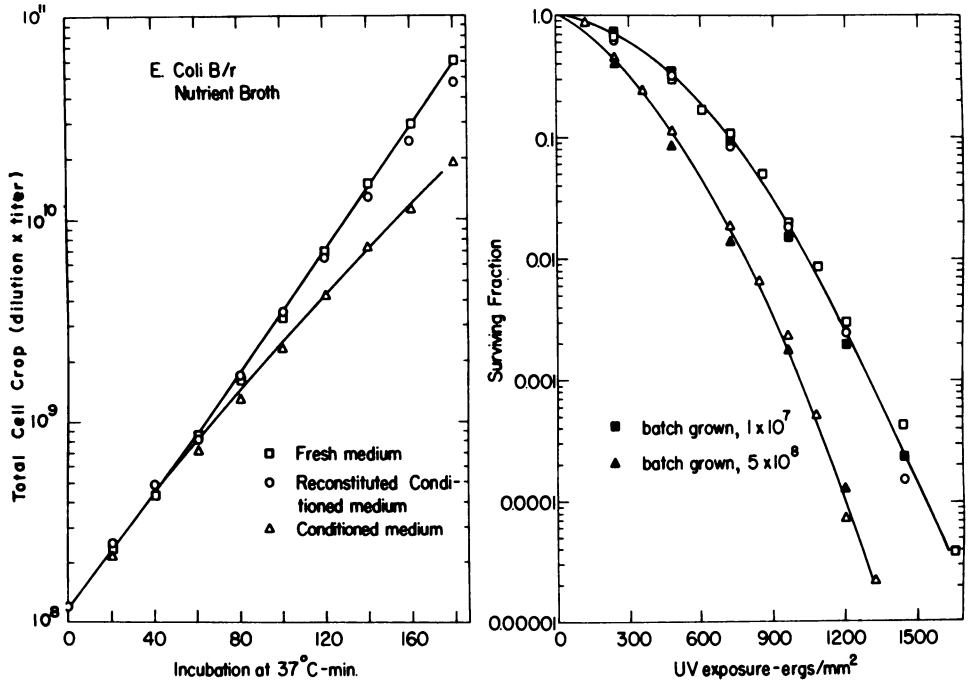

FIG. 5. *UV sensitivity and growth of E. coli B/r in conditioned Nutrient Broth medium with balanced growth procedures. The left panel shows the growth of three cultures all kept between 10^7 and 2×10^7 cells/ml. These cultures were started at 10^7 cells/ml by diluting a growing culture at 2×10^7 cells/ml with an equal volume of medium. Dilution was then continued every 10 min with fresh medium* (□), *conditioned medium* (△), *or reconstituted, conditioned medium* (○). *The details of medium preparation are given in the text. The right panel shows the UV survival of cells at the end of the growth period defined in the left panel. In addition, the UV survival of E. coli B/r cells batch grown to 10^7 cells/ml and 5×10^8 cells/ml are shown for comparison* (■, ▲).

occurred at a cell titer of about 10^7 to 2×10^7 cells/ml. If the concentration of glucose was reduced so that growth was limited to about this value (from 0.05 to 0.001% glucose), a similar period of increased UV sensitivity still occurred, just before the cells entered stationary phase because the glucose was exhausted (Fig. 7). This is consistent with the hypothesis that the period of increased UV sensitivity is due to exhaustion of nutrients and is not due to the accumulation of a sensitizer during growth (which can be counteracted or neutralized by fresh nutrients).

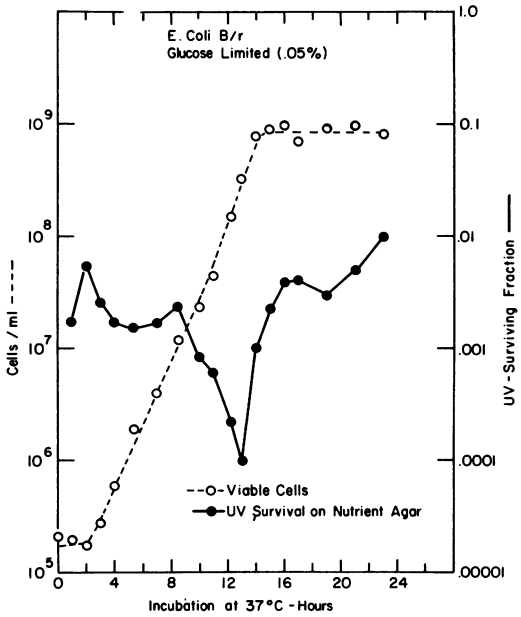

FIG. 6. *UV survival of E. coli B/r after a constant dose of 960 ergs/mm² as a function of growth in glucose-limited, minimal medium. The cells were diluted into buffer before irradiation and afterwards were plated on Nutrient Broth agar.*

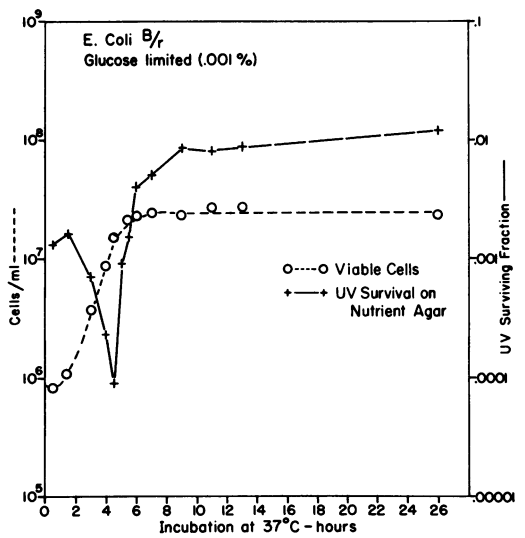

FIG. 7. *UV survival of E. coli B/r after a constant dose of 960 ergs/mm² as a function of growth in a glucose-limited, minimal medium. Irradiated cells were plated on Nutrient Broth agar. The growth was limited to 2.5 × 10⁷ cels/ml by the amount of glucose available.*

DISCUSSION

Our results indicate that there are significant changes in the UV sensitivity of *E. coli* B/r during batch culture growth, that they are associated with changes in the shoulder of the UV survival curve, that they also may be correlated with differences in the postirradiation division delay, and that they are largely absent from the repair-deficient mutant B_{s-1}. On the other hand, we have observed a complex pattern of fluctuations in the sensitivity of *E. coli* B which is believed to be mutant at the *lon* locus. There are two components of these changes in sensitivity: first, the *rising* sensitivity that occurs somewhat prior to the onset of the stationary phase, and which appears from our experiments to be brought about by depletion of nutrients in the growth medium; and, second, the decreasing sensitivity as the culture enters stationary phase. We have done no experiments to clarify further the basis of this latter component, but it may be associated with the unique properties of nongrowing, stationary-phase cells. A further important phase of the growth cycle, about which we have no data on UV sensitivity, is the lag phase.

The phenomena described in this paper are closely paralleled by the changes in X-ray sensitivity in cultures of *E. coli* B/r described by Stapleton (15) and by Adler and Hardigree (1). They observed similar periods of altered sensitivity, but in these cases the parameter that varied during growth was the *slope* of the X-ray survival curves. The changes in X-ray and UV sensitivity are superficially similar to changes in the luminescence emitted by growing cultures of *P. fischeri*, reported by Kempner and Hanson (13). In this case, a period of decreased luminescence would be analogous to our period of decreased UV resistance. They interpreted this phase of low luminescence as due to an inhibitor of luciferase which was present in the growth medium. Cells inoculated into fresh medium were supposed to lose their luminescent ability gradually but at the same time to metabolize the inhibitor, thus eventually returning to normal levels of luminescence.

It seems reasonable to believe that the underlying mechanism involved in the changes in X-ray and UV resistance in *E. coli* B/r are similar (9).

A question of primary interest is whether these changes in sensitivity are intrinsic to the individual cells themselves, or are the result of changes in the population of cells. For example, it might be argued that the age distribution of cells changes as the population approaches stationary phase, and that if the UV (or X-ray) sensitivity depends on the age since the last division the net sensitivity of the population would change. Such an explanation would demand that the variations in UV (or X-ray) sensitivity with cell age be at least as great (and probably much greater) as the changes observed in the population. It would appear that this argument can be rejected because the fluctuations in the UV sensitivity of synchronized *E. coli* cells are not sufficiently great to account for the sensitivity changes observed in our experiments (12). More generally, of course, it is difficult to dispose rigorously of the question of population changes as opposed to uniform cellular changes. However, as will be argued below, it is possible to explain both the variations in UV and X-ray sensitivity in a simple and reasonable manner if we assume that a single cellular property changes during batch-culture growth.

The experiments with postirradiation division delay and with the repair-deficient mutant B_{s-1} strongly suggest that the property in question is the capacity to repair DNA damage. There is evidence (8-11) suggesting that the repair of both UV and X-ray damage, as well as other kinds of damage, may involve common enzymatic steps, and on this basis the close parallel between variations in the sensitivity to these two agents is easily explained. Further, the shape of the survival curves measured at different times during the growth cycle is consistent with this explanation. For example, the variation in UV sensitivity could not be due to changes in intercellular shielding of the "target," for if such were the case the slope of the survival curve should vary.

If we accept these arguments, our results indicate that growth of *E. coli* B/r in a medium partially depleted of nutrients causes a decreased capacity for repair of DNA damage (both UV and X-ray) and that something in fresh medium reverses or neutralizes the effect of depleted medium. That the effect of depleted medium does not occur through the accumulation of an inhibitor is indicated by the data in Fig. 6 and 7 on glucose-limited growth. The results can be most concisely described by saying that there is some "protective" material in fresh media which is metabolized by the growing cells. It seems unreasonable to believe that this protective material is any particular chemical substance or even a special class of substances present in the several kinds of media we have used. Furthermore, it is difficult to see how the results with glucose-limited media could be explained by assuming that the protective agent is a contaminant of some component of the media. Rather, it seems simpler and more reasonable to conclude that the nutrients themselves constitute the "protective" materials in the media.

We are inclined, therefore, to suggest that the intercellular concentration (or activity) of the "repair enzyme(s)" varies during batch-culture growth in response to changes in cellular metabolism caused by the changing environment. Variations of cellular enzymes during culture growth is a well-known phenomenon [for example, see the summary by Dean and Hinshelwood (5)]. It is an important part of this hypothesis that the photochemical damage caused by UV is for all practical purposes independent of the growth phase of the culture. Ginsberg and Jagger (7) observed differences in UV sensitivity between log-phase and stationary-phase *E. coli* $T^-A^-U^-$ similar to those reported here. From experiments on the photoreactivity, they concluded that the initial UV damage was similar for the two stages of growth.

The explanation we offer for the fluctuations in UV (and X-ray) sensitivity is different in principle from that given by Kempner and Hanson (13) for the similar changes in luminescence of *P. fischeri*. They interpreted these changes as being due to a dialyzable inhibitor of luciferase present in the nutrient medium but metabolized by the growing cells, so that after a time luminescence returns. An analogous explanation for the fluctuations in UV sensitivity of *E. coli* B/r is not consistent with the data presented. For example, it would predict that a growing culture continually diluted with fresh medium should become UV sensitive because the inhibitor of the repair enzyme(s) could never be completely metabolized. Further, it would predict that dilution of the culture with conditioned medium (which does not contain the inhibitor) would cause the cells to become UV-resistant. Neither prediction is confirmed by our experiments.

As a corollary to our hypothesis, we would suggest a close relationship between the intracellular activity of the dark repair enzymes and the magnitude of the shoulder of the UV survival curve. From the data of Stapleton (15), it would also follow that the slope of the X-ray survival curve would (with respect to repair phenomena) be related to the UV curve shoulder, at least in *E. coli* B/r [*see also* Haynes (9, 10)]. Finally, if the repair enzymes are not specific for UV damage, but act on that because of other agents [for example, nitrogen mustard (8-10)], then the sensitivity to these agents should vary in a manner similar to that shown in Fig. 1.

ACKNOWLEDGMENT

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