Photochemistry and Photobiology Vol. 33, pp. 203 to 210

0031-8655/81/0201-0203$02.00/0

MEMBRANE DAMAGE CAN BE A SIGNIFICANT FACTOR IN THE INACTIVATION OF *ESCHERICHIA COLI* BY NEAR-ULTRAVIOLET RADIATION

STEPHEN H. MOSS* and KENDRIC C. SMITH

Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305, USA

(*Received* 5 *May* 1980; *accepted* 3 *September* 1980)

Abstract—A DNA repair competent strain of *Escherichia coli* K-12 showed sensitivity to inorganic salts (at concentrations routinely used in minimal media) after irradiation with broad spectrum near-UV radiation, at fluences that caused little inactivation when plated on complex growth medium. This effect was not observed with cells that had been exposed to 254 nm radiation. This sensitivity to minimal medium was increased by increasing the salt concentration of the medium and by increasing the pH of the medium. This sensitivity was greatly increased by adding to the medium a low concentration of commercial glassware cleaning detergent that had no effect on unirradiated cells or far-UV irradiated cells. These findings may explain the large variability often observed in near-UV radiation survival data, and demonstrate that, at least on minimal medium plates, membrane damage contributes significantly towards cell killing. This phenomenon is largely oxygen dependent.

INTRODUCTION

While other chemical and physiological effects of far-UV radiation (i.e. 254 nm) have been described, DNA damage has been found to be of overriding importance in cell killing (see Swenson, 1976, for review). In contrast, the photochemical effects of UV radiation of wavelengths greater than 320 nm (near-UV) are more evenly distributed throughout the cell. As well as producing lesions directly in DNA, near-UV radiation causes damgae to DNA repair enzymes, cell membranes and metabolic systems (see Webb, 1977, for review), with some effects, e.g. growth delay, occurring at essentially nonlethal fluences (Ramabhadran and Jagger, 1976). Although the multiple effects of near-UV radiation are well known, and although early work suggested that the lethal effects of near-UV radiation were associated with physiological rather than genetic damage (Hollaender, 1943), the current interpretation of near-UV radiation survival curves is generally based on the assumption that inactivation is due to DNA damage (Webb and Brown, 1976; Webb *et al.*, 1976), in some cases amplified by damage to DNA repair enzymes (Tyrrell and Webb, 1973; Tyrrell *et al.*, 1973; Tyrrell, 1976).

It is the purpose of this report to reinvestigate the mechanism of inactivation of *Escherichia coli* by near-UV radiation. We observed that, after near-UV irradiation, the survival of repair competent strains of *E. coli* was significantly decreased when viability was assessed on minimal growth medium, as compared with nutrient agar or other complex growth media. This contrasts with the survival of wild-type strains after far-UV irradiation, which was largely independent of the post irradiation growth media. Furthermore, suvival curves after near-UV irradiation are subject to more variation between experiments than survival curves obtained after far-UV irradiation (Peak, 1970). This variation has led workers to select, often empirically, very specific conditions, e.g. stage growth phase, in order to improve reproducibility (Tuveson and Jonas, 1979). The fact that this variability in sensitivity to near-UV radiation is not readily explainable in terms of DNA damage and the recent report that the genetic control of near-UV radiation sensitivity is independent of far-UV radiation sensitivity (Tuveson and Jonas, 1979), have led us to suspect that the inactivation of cells by near-UV radiation is not solely by damage to DNA. We find that on minimal medium plates, membrane damage contributes significantly to cell killing by near-UV radiation, a process that is largely oxygen dependent.

*On sabbatical leave from the School of Pharmacy and Pharmacology, University of Bath, Bath, Avon, England.

MATERIALS AND METHODS

Bacterial strain and culture conditions. Strain SR385 is *E. coli* K-12 strain JG139 F⁻ *rha*-5 *lacZ*53 *rpsL*151 *thyA*36 *deo*(*C*2?), which was obtained from E. C. Friedberg. Stationary phase cultures were grown at 37°C in a shaking water bath in 50 m/ flasks containing 20 m/ of minimal growth medium. Inoculation was always with 0.2 m/ of an overnight culture containing 2×10^9 colony units (CFU) per m/, and cells were always harvested after 24 h of growth. This procedure was adhered to in order to minimize variation in sensitivity and photoreactivability, as previously demonstrated (Tyrrell *et al.*, 1972a, b). Cells were harvested on membrane filters (Millipore Corp., HA, 0.45 μm pore size), and resuspended in 0.067 *M* $NaKPO_4$ buffer, pH 7.0 (Castellani *et al.*, 1964), at a concentration of 10^7 CFU/m/. This suspension was used for irradiation.

Media. The minimal medium used was composed of: 4×10^{-2} *M* K_2HPO_4, 1.5×10^{-2} *M* KH_2PO_4, 4.1×10^{-4} *M* $MgSO_4$, 7.6×10^{-3} *M* $(NH_4)_2SO_4$, 1.4×10^{-3} *M* sodium citrate, 3.4×10^{-5} *M* $CaCl_2$,

9.0×10^{-7} *M* $FeSO_4$, 0.4% glucose, 10 μg/m/ thymine and 0.5 μg/m/ thiamine·HCl. This is based on a minimal medium originally described by Davis and Mingioli (1950), and subsequently modified by Lederberg (1950), Kaplan *et al.* (1962) and Ganesan and Smith (1968). Where specified, the concentration of inorganic salts in this growth medium was increased twofold or decreased tenfold and in the latter case was, on occasion, supplemented with 100, 200 or 300 m*M* sodium chloride. For the purposes of preparing plates, the minimal media described above were solidified using 1.6% Noble agar (Difco). This agar was used since less pure agars have been shown to inhibit some branches of DNA repair (Van der Schueren *et al.*, 1974). The complex medium was composed of nutrient broth (Difco, 8 g//) and yeast extract (Difco, 7.5 g//). The complex medium plates were composed of nutrient agar (Difco, 23 g//) and yeast extract (Difco, 7.5 g//). After pouring, the plates were stored at room temperatures for 2 days, and then stored at 4°C for up to 7 more days before use. During preparation and storage, plates were only exposed to light from 'gold' fluorescent lamps (General Electric) to prevent the formation of toxic photoproducts in the medium by white fluorescent light (Webb and Lorenz, 1972).

Far-UV irradiation. An 8W General Electric germicidal lamp, emitting primarily at 254 nm, was situated at a distance of 41 cm from the bacterial suspension. Samples (10 m/ in a 9.0 cm-inside-diameter glass Petri dish bottom) were irradiated on a rotary shaker (Eberbach Corp.). The far-UV radiation fluence rate was ~ 1.1 W/m^2, and was checked before each experiment with a germicidal photometer (International Light, Inc., no. IL-254) whose calibration was verified by uranyl oxalate actinometry (modified from Bowen, 1946). No correction for cell masking was required at the bacterial suspension concentration used (10^7 CFU/m/).

Near-UV irradiation. Six General Electric BLB 15T8 'black light-blue' lamps were mounted as two vertical banks of three lamps. The emission of these lamps is chiefly between 320 and 405 nm (Jagger, 1967). Samples (5 m/) of bacterial suspensions in 0.067 *M* phosphate buffer in 15 × 1.5 cm glass tubes (Kimax) were held vertically, spaced 1.5 cm apart and centered between the two banks of fluorescent lamps. The distance between the front surfaces of the two banks of lamps was 4 cm. The construction of the apparatus included a fan at one end, and channels for the insertion of a double metal shutter from the other end. Samples in the positions used were found to remain at 25° ± 1°C. When not in use, positions were fitted with tubes containing 5 m/ of water to keep reflected light constant. For 15 min prior to and during near-UV irradiation, samples were bubbled with either humidified air or nitrogen (Liquid Carbonic 99.999% purity). Except for the two outer positions at each end, the nine remaining tubes were found (from survival curves) to receive equal fluence rates. For an approximate dosimetric comparison with published results (e.g. Brown and Webb, 1972), a survival curve was obtained using our apparatus for *E. coli* K-12 *uvrA uvrB recA phr* (SR362). This strain has an exponential survival curve under our conditions, and was inactivated to a surviving fraction of 1.0×10^{-5} by 15 min of near-UV irradiation.

Assessment of survival. For control and irradiated samples, 0.2 m/ samples were serially diluted in 0.067 *M* phosphate buffer, and 0.2 m/ of the final dilution was spread onto the surface of each of three plates. Where survival on more than one type of plating medium was investigated, three plates of each medium were used. The colonies were counted after incubation at 37°C for 48 h (complex medium) or 72 h (minimal media). In those experiments involving postirradiation holding in liquid media, control and irradiated samples were diluted before holding to give an initial total cell concentration of 5×10^4 cells/m/ in 20 m/ of holding medium, which had been prewarmed to 37°C. These media were then shaken in a water bath at 37°C, and samples were removed and plated at various time intervals. The surviving fraction was calculated by dividing the number of CFU in the treated sample by the number of CFU in the unirradiated sample at time zero. All manipulative procedures were carried out at room temperatures under 'gold' fluorescent lamps (General Electric).

RESULTS

The survival of stationary phase cells of *E. coli* SR385 after near-UV and far-UV irradiation, assessed on either minimal or complex medium, is shown in Fig. 1. The survival curves after far-UV irradiation show little difference when plated on complex or

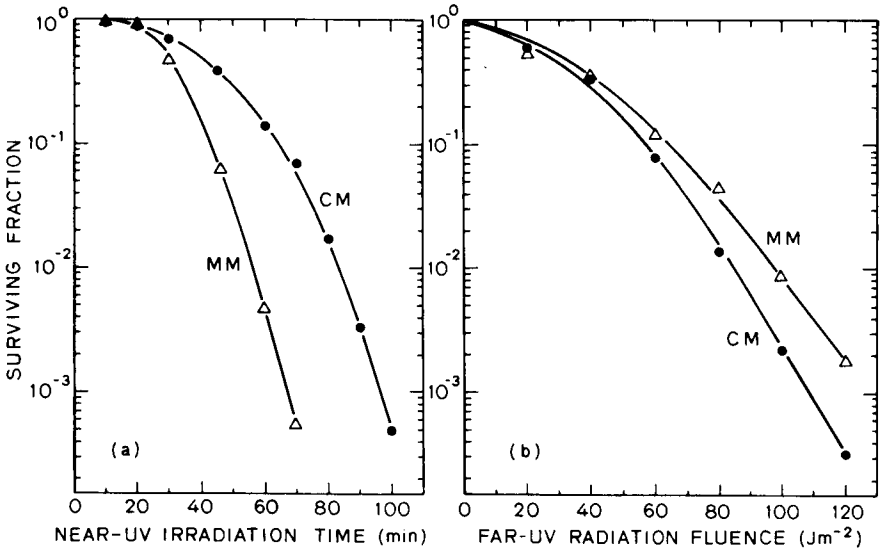

Figure 1. Survival curves for wild-type *E. coli* K-12 (SR385) after (a) near-UV, and (b) far-UV irradiation. Viability was assessed using either complex medium (CM) plates (●) or minimal medium (MM) plates (△).

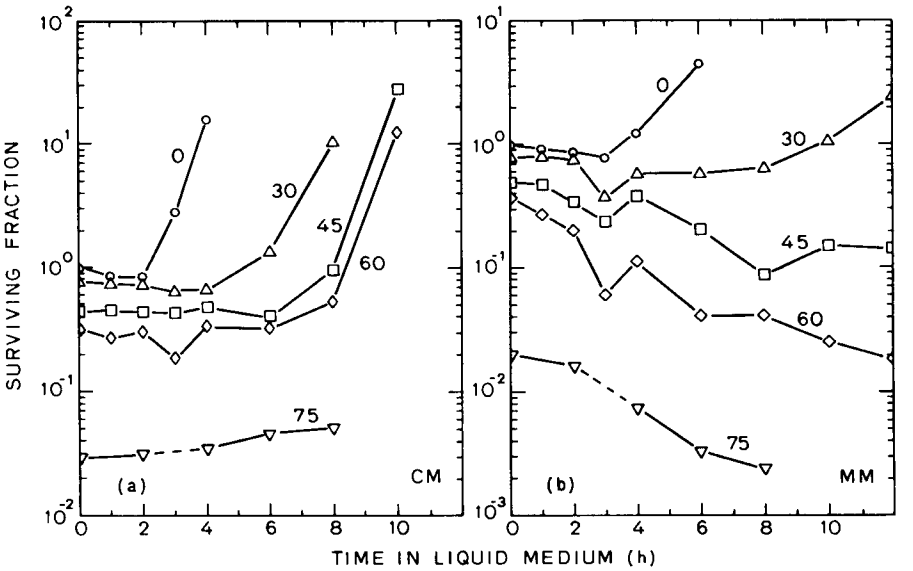

Figure 2. The surviving fraction of *E. coli* K-12 (SR385) irradiated with a range of near-UV radiation fluences, and then held at 37°C in (a) liquid complex medium, or (b) liquid minimal medium for the times indicated on the abscissa before plating on complex medium. Symbols: ○, zero; △, 30; □, 45; ◇, 60; ▽, 75 min of near-UV irradiation.

minimal growth medium. After near-UV irradiation, however, this strain shows increased sensitivity when plated on minimal medium, a response that was also observed during preliminary experiments with other wild-type strains.

In order to investigate the nature of near-UV radiation sensitivity on minimal growth medium, an experimental protocol was adopted whereby, after irradiation, samples of the irradiated and unirradiated cells were transferred to liquid medium, either minimal or complex and then, after incubation at 37°C for various times, the viability was assessed by plating on complex medium. This protocol was adopted to determine the time course of the inactivation in minimal medium, and to enable variations in the minimal growth medium formulation to be made easily without the need for preparing separate batches of minimal medium plates.

The effects of holding suspensions of *E. coli* SR385, exposed to different fluences of near-UV radiation, in liquid minimal medium or in liquid complex medium for various times before plating on complex medium, are shown in Fig. 2. Unirradiated cells held in complex medium showed a small growth delay, expected as they were harvested in stationary phase. Near-UV irradiated cells held in complex medium exhibited a longer growth delay. Unirradiated cells held in minimal medium behaved similarly to those held in complex medium. Cells irradiated with near-UV radiation, at fluences that resulted in relatively little inactivation if they were plated immediately on complex medium showed, over a time scale of several hours, a progressive decrease in viability in minimal medium. The magnitude of this decrease in viability in liquid minimal medium (Fig. 2b) approximated the difference in survival obtained by plating the cells on complex medium versus minimal medium immediately after irradiation (Fig. 1a). For subsequent experiments, a single irradiation time (60 min) was chosen, which produced a significant decrease in survival in liquid minimal medium relative to complex growth medium.

One of the differences between minimal and complex medium is the presence of relatively high concentrations of inorganic salts in the former. The minimal medium used in this study contains inorganic salts with a total molarity of 64 m*M* (see Methods). Other frequently used minimal media contain up to 160 m*M* of inorganic salts (Neidhardt, 1974). As a first stage in our studies, it was decided to vary the concentration of inorganic salts in our minimal medium, either by a twofold increase or a tenfold decrease, while leaving the glucose, thymine and thiamine concentration unaltered. The results obtained by holding unirradiated and near-UV irradiated *E. coli* SR385 cells in these altered minimal media before plating on complex media are shown in Fig. 3. In the diluted minimal medium there was little or no decrease in survival during holding, but in the more concentrated minimal medium the rate and extent of the decrease in survival was accentuated.

In order to determine if the effects of salt concentration were a property of the particular inorganic salts in the formulation of the minimal medium, a similar experiment was performed in which the tenfold diluted minimal medium was supplemented with sodium chloride at various concentrations (Fig. 4a and Fig. 4b). In order to confirm the specificity of this phenomenon for near-UV irradiated cells, a parallel experiment was performed using far-UV irradiated cells (Fig. 4c). The addition of sodium chloride at 100 m*M* produced a decrease in the survival of

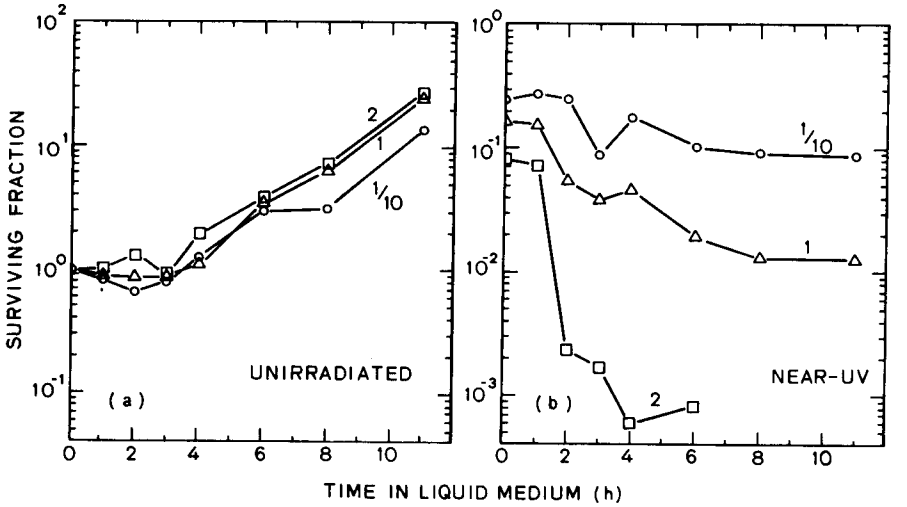

Figure 3. The surviving fraction of *E. coli* K-12 (SR385) in different holding media, (a) unirradiated and (b) after 60 min of near-UV irradiation. Samples were held at 37°C in minimal medium with tenfold-diluted salts (○), single strength minimal medium (△), or minimal medium with double strength salts (□) for the times indicated on the abscissa before plating on complex medium.

near-UV irradiated cells approximately equivalent to that produced by single strength minimal medium. Sodium chloride at a concentration of 200 m*M* produced an effect of a similar magnitude to twofold concentrated minimal medium, and this effect was increased further at 300 m*M* sodium chloride. For far-UV irradiated cells and unirradiated cells, the 300 m*M* sodium chloride resulted in a decreased viability at the longer time intervals, but the lower concentrations, i.e. 100 m*M* and 200 m*M*, had no observable effect other than a slightly reduced growth rate.

In order to establish that the effects of holding in liquid minimal medium, described above, correspond to the decreased survival seen on minimal medium plates (Fig. 1a), full survival curves after near-UV and far-UV irradiation were carried out, plating immediately after irradiation on complex medium and on minimal medium containing one tenth the normal concentration of inorganic salts, with or without 200 m*M* sodium chloride (Fig. 5). The reduced inorganic salt concentration was sufficient to permit colony formation, but did not result in decreased survival. The presence of 200 m*M* sodium chloride (Fig. 5a) mimicked the decreased survival seen on single strength minimal medium after near-UV irradiation (Fig. 1a).

Effect of anoxia during near-UV irradiation

The preceding experiments were carried out using suspensions bubbled with air during near-UV irradiation. As it is known that inactivation by

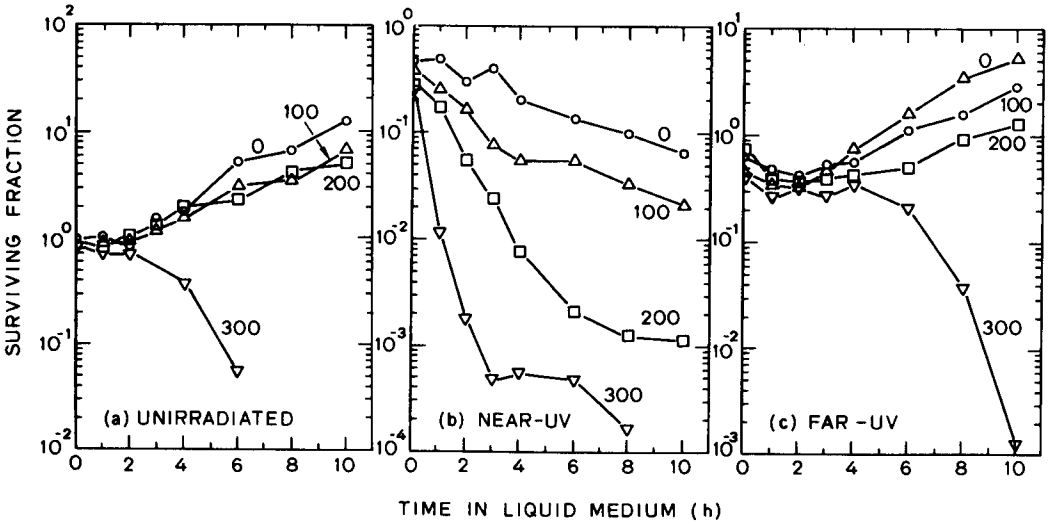

Figure 4. The surviving fraction of *E. coli* K-12 (SR385), (a) unirradiated, (b) after 60 min of near-UV irradiation, and (c) after 33 Jm^{-2} of far-UV radiation. Samples were held at 37°C in minimal medium with tenfold-diluted salts (○), or in minimal medium with tenfold-diluted salts with sodium chloride added at 100 m*M* (△), 200 m*M* (□), or 300 m*M* (▽) for the times indicated on the abscissa before plating on complex medium.

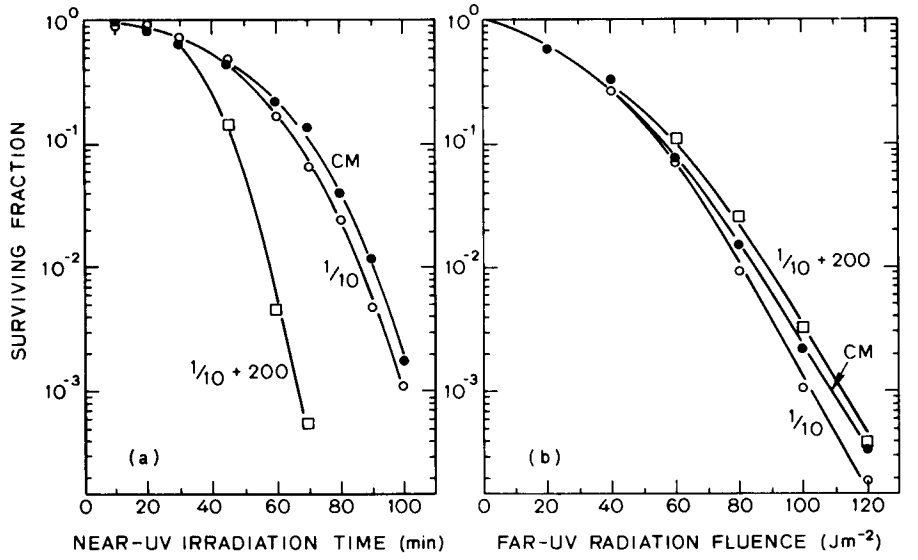

Figure 5. Survival curves for *E. coli* K-12 (SR385) after (a) near-UV and (b) far-UV irradiation. Viability was assessed using plates made from complex medium (●), minimal medium with tenfold-diluted salts (○), and minimal medium with tenfold-diluted salts with 200 m*M* sodium chloride added (□).

near-UV radiation is decreased in the absence of oxygen (Webb and Lorenz, 1970; Webb and Brown, 1979), the influence of oxygen during near-UV irradiation was tested in our system. The lethality was greatly reduced, and the sensitivity to high concentrations of salt was still observed in the absence of oxygen (Fig. 6).

Factors in media preparation effecting sensitivity to near-UV radiation

During preliminary experiments, considerable variation, equivalent to about a 25% change in the fluence, was observed in survival curves after near-UV irradiation. While the careful control of preirradiation growth conditions reduced the variation observed on complex medium, large variations were still observed on minimal medium (data not shown). In view of the preceding results, it was thought that some of this variation could be due to localized changes in salt concentrations at the surface of minimal medium plates, brought about by evaporation and/or condensation during plate preparation and/or storage. However, occasional variations, associated specifically with the use of different batches of plates, were larger than could be expected from this cause. In extreme cases, a fluence that produced no inactivation on complex medium resulted in surviving fractions from 10^{-1} to 10^{-4} on different batches of minimal medium. In an attempt to determine the source of this variation between batches of plates, a number of possible factors in the method of preparation of the media were investigated.

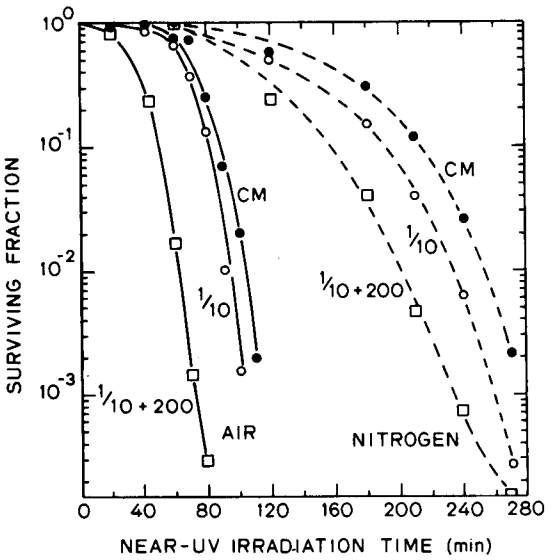

Figure 6. Survival curves for *E. coli* K-12 (SR385) after near-UV irradiation in the presence of air (solid lines) or nitrogen (broken lines). Viability was assessed using plates made from complex medium (●), minimal medium with tenfold-diluted salts (○) and minimal medium with tenfold-diluted salts with 200 m*M* sodium chloride added (□).

Changes in the pH of the liquid minimal medium used to hold the cells after irradiation resulted in changes in survival. Over the range from pH 6.6 to 7.4, a significant decrease in survival was observed (Fig. 7). Similar changes were observed when mininmal medium plates were prepared having different pH values (results not shown). However, these changes in pH were outside the variation expected during routine preparation of media, and the effect produced, although significant, was less than the variation occasionally observed between batches of plates.

The largest effect on the survival of near-UV irradiated *E. coli* SR385 cells was produced by including a commercial cleansing agent (Aura, Calgon Corporation), used in this laboratory for the machine washing of glassware, in the minimal growth medium. At a concentration of 1 μg/mℓ, this cleansing agent con-

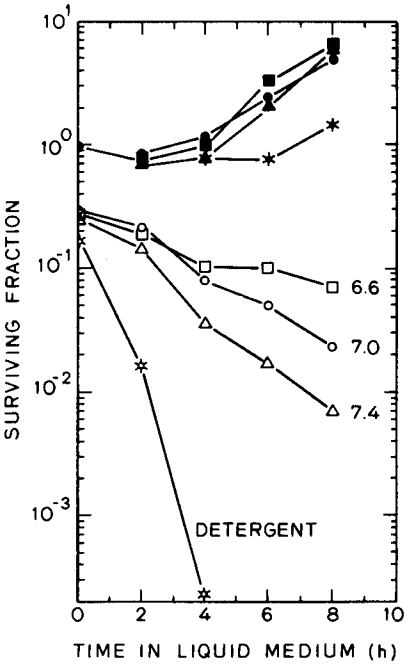

Figure 7. The surviving fraction of *E. coli* K-12 (SR385) unirradiated (solid symbols) or iradiated for 60 min with near-UV radiation (open symbols). The samples were held at 37°C in minimal medium at pH 6.6 (□, ■), pH 7.0 (○, ●) or pH 7.4 (△, ▲), or in minimal medium containing 1 μg/m*l* of commercial detergent (✱, ✱), for the times indicated on the abscissa before plating on complex medium.

siderably reduced the survival of near-UV irradiated cells in minimal medium, but did not significantly reduce the viability of unirradiated cells (Fig. 7), or far-UV irradiated cells (data not shown). As a result of this observation, the concentration of cleansing agent used for washing glassware was reduced, and the rinsing cycle was extended. This procedure has apparently succeeded in eliminating variations between batches of minimal medium plates.

DISCUSSION

Our results show that the increased sensitivity of *E. coli* to near-UV radiation, observed when survival is assessed on minimal medium plates versus complex medium plates (Fig. 1a), is due to the sensitivity of the irradiated cells to the concentration of inorganic salts present in the medium (Fig. 3b). Near-UV radiation sensitivity also occurred in minimal medium in which the inorganic salts were diluted tenfold, and to which sodium chloride was added at either 100 m*M* or 200 m*M* (Fig. 4b); conditions under which unirradiated (Fig. 4a) or far-UV irradiated (Fig. 4c) cells showed no sensitivity.

Sensitivity to inorganic salts is a characteristic of cells damaged by mild heating in aqueous environments. Clark and Ordal (1969), studying the effect of different media on the enumeration of *Salmonella typhimurium* heated at 48°C, observed a higher viability for heated cells on a complex medium (Trypticase Soy Broth) than on a medium (Levine Eosin Methylene Blue Agar) containing 2% sodium chloride (342 m*M*). The difference between the survival on the complex medium and that containing sodium chloride has been associated with 'sublethally injured' cells, and the lesions have been identified as a loss of membrane integrity, observed as the leakage of cell components that absorb at 260 nm (Iandolo and Ordal, 1966; Russell and Harries, 1967). More specifically, recovery from salt sensitivity has been linked to lipid biosynthesis (Tomlins *et al.*, 1972), and the loss of magnesium has been identified as an event closely linked to heat inactivation (Hurst *et al.*, 1974). It should be pointed out that it is not entirely clear as to the relationship between cause and effect, e.g. Grau (1978) found that the quantitative effects of heat on membrane transport systems and viability in *E. coli* did not suggest the former as the only cause of inactivation. In general, however, the evidence is that heat-induced salt sensitivity, i.e. enhanced inactivation on media with relatively high concentrations of salt, is associated with membrane damage. By analogy with the 'sublethal injury' described after heat treatment, we suggest that the increased lethality of near-UV irradiated cells on minimal medium (with a high salt concentration), compared with that on complex medium, is due to the death of cells with damaged membranes. This is supported by the increased sensitivity of near-UV irradiated cells to a glassware cleaning agent (Fig. 7); sensitivity to detergents being a general characteristic of membrane damage.

If the hypothesis is made that the *increased sensitivity* of near-UV irradiated cells on high salt media is due to membrane damage, then near-UV radiation survival curves obtained on high salt media reflect inactivation by membrane damage and by other causes (e.g. DNA lesions). Near-UV radiation survival curves obtained on complex medium, however, may reflect inactivation only by DNA lesions, although it is possible that, at the higher near-UV radiation fluences where inactivation is observed even on complex medium, more severe membrane damage may play a part in inactivation even on complex medium. If, however, as a first approximation, it is assumed that membrane damage plays no part in inactivation on complex medium, then a fluence response for the membrane component of inactivation may be obtained by expressing the survival on high salt medium as a fraction of the survival on complex medium. The 'corrected surviving fractions', derived in this manner from the data in Fig. 6, are shown in Fig. 8. Although the absolute slopes of these lines will be dependent on the concentration of salt chosen, for a given salt concentration a comparison between the curves obtained for air and nitrogen will yield a value for the sensitization by oxygen of near-UV radiation-produced membrane damage. The value obtained from Fig. 8 for the oxygen sensitization of membrane damage is ~ 3.5. This should be compared with the

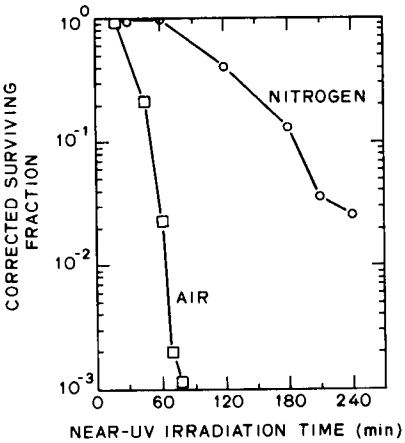

Figure 8. Fluence response for the inactivation of near-UV irradiated cells of *E. coli* K-12 (SR385) by presumptive membrane damage. The results are shown for near-UV irradiation under air (□), or under nitrogen (○). The 'corrected surviving fraction' is derived from the data in Fig. 6 by expressing the surviving fraction on minimal medium with tenfold-diluted salts and 200 m*M* sodium chloride added, as a fraction of the surviving fraction on complex medium. The rationale for this calculation is described in the Discussion.

value of ~ 2.5 for the oxygen sensitization of presumptive DNA damage, as determined from a comparison of the survival curves obtained on complex medium (Fig. 6).

Published data on near-UV radiation-produced membrane damage have not been interpreted as "convincing evidence that such damage contributes to near-UV lethality under usual growth conditions" (Webb, 1977, p. 214), although Webb does mention the inactivation of cells in physiological saline that was observed by Hollaender (1943) after near-UV irradiation. However, in view of the results reported here, the published evidence for lethality from near-UV radiation-produced membrane damage requires reexamination.

Significant inactivation of several membrane transport systems in *E. coli* by sublethal fluences of near-UV and visible radiation was observed by Sprott *et al.* (1976), who concluded, from the differential wavelength dependence of the systems studied, that the effect was not one of generalized permeability. They also concluded that four photosensitizers were involved, three active under aerobic conditions and one in the absence of oxygen. Supporting the multiple actions of near-UV radiation, Koch *et al.* (1976), investigating the effect of near-UV radiation on galactoside transport in *E. coli*, found that the permease and the metabolic energy systems could be inactivated independently by near-UV radiation. Robb *et al.* (1978) measured the action spectra for two separate leucine transport systems in *E. coli*, and also concluded that the observed effects were not due to generalized permeability. The two peaks in both action spectra at 290 nm and 365 nm suggest there may be two distinct chromophores, or one chromophore with two absorption bands. To date no chromophores for membrane transport inactivation have been identified. Work is in progress to measure wavelength dependence of the near-UV radiation-produced salt sensitivity reported here.

Near-UV irradiated *rel*$^-$ and ^{4}Srd$^-$ mutants of *E. coli* (strains NC52 and RJ-1, respectively, provided by Dr J. Jagger) showed salt sensitivity similar to that seen with the wild-type strain used in this study (data not shown). Thus, it does not appear that the rare *t*-RNA base 4-thiouracil, which has been shown to be responsible for near-UV radiation produced growth delay (Rhamabhadran and Jagger, 1976), is the chromophore involved in near-UV radiation-produced salt sensitivity. Similarly, in experiments with a near-UV radiation sensitive mutant of *E. coli* (strains RT-2 *nur*$^-$ and RT-4 *nur*$^+$ provided by Dr R. W. Tuveson), both the *nur*$^+$ and *nur*$^-$ strains showed increased sensitivity on minimal medium after near-UV irradiation (data not shown). These results indicate that the *nur* gene is not directly linked to salt sensitivity after near-UV irradiation.

In addition to the main observations, several factors arising from experimental procedure, which affected the observed near-UV radiation sensitivity, were noticed. Minor changes in the pH of the minimal medium and the presence of a commercial glassware cleansing agent (Fig. 7) have been shown to affect near-UV radiation sensitivity. It was also noticed that at fluences resulting in low surviving fractions, i.e. below 10^{-4} (data not shown), viable counts on minimal media were higher than would be predicted from an extrapolation of the exponential inactivation observed at intermediate survival levels. The larger number of inactivated cells present in the samples plated, undiluted in order to detect the smaller number of survivors, may protect the other cells against inactivation by the inorganic salts. Consistent with this hypothesis, protection was gained by the addition of Casamino Acids to our minimal medium (results to be published separately). Lastly, it was noticed that near-UV irradiated cells, diluted and spread onto solid medium after holding in liquid medium (either complex or minimal) for approximately 3 h after irradiation, showed additional inactivation (see, e.g. Fig. 2b). As this occurs even when transferring from liquid complex medium to solid complex medium, it is suggested that it was either a function of the physical stress on the cells during dilution or of spreading them on the plates, and may reflect a weakness in the cell envelope at that time.

In conclusion, the following factors relate to the inactivation of *E. coli* by near-UV radiation. (1) The increased sensitivity of near-UV irradiated cells on minimal medium is due to the concentration of inorganic salts present. (2) This sensitivity of inorganic salts most probably reflects membrane damage. (3) The presumed membrane damage is formed by near-UV irradiation under aerobic conditions, and to a lesser extent under anaerobic conditions. (4) Sensi-

tivity and resistance to membrane damage may explain published differences in near-UV radiation sensitivity that are not related to far-UV radiation sensitivity (e.g. Tuveson and Jonas, 1979). (5) This form of damage should be considered especially in the interpretation of experiments using minimal medium plates (e.g. the scoring of mutations) and experiments where other factors may contribute to membrane damage (e.g. interaction between mild heat and near-UV radiation). (6) Trace contamination of glassware with cleaning agents should be carefully avoided.

Acknowledgements—This work was supported by U.S. Public Health Service research grants CA02896 and CA-06437, and program project grant CA-10372 from the National Cancer Institute, DHEW.

REFERENCES

Bowen, E. J. (1946) In *The Chemical Aspects of Light*. Clarendon Press, Oxford.
Brown, M. S. and R. B. Webb (1972) *Mutat. Res.* **15**, 348–352.
Castellani, A., J. Jagger and R. B. Setlow (1964) *Science* **143**, 1170–1171.
Clark, C. W., and Z. J. Ordal (1969) *Appl. Microbiol.* **18**, 332–336.
Davis, B. D. and E. S. Mingioli (1950) *J. Bacteriol.* **60**, 17–28.
Ganesan, A. K. and K. C. Smith (1968) *J. Bacteriol.* **96**, 365–373.
Grau, F. H. (1978) *Appl. Environ. Microbiol.* **36**, 230–236.
Hollaender, A. (1943) *J. Bacteriol.* **46**, 531–541.
Hurst, A., A. Hughes, D. L. Collins-Thompson and B. G. Shah (1974) *Can. J. Microbiol.* **20**, 1153–1158.
Iandolo, J. J. and Z. J. Ordal (1966) *J. Bacteriol.* **91**, 137–142.
Jagger, J. (1967) In *Introduction to Research in Ultraviolet Photobiology*, pp. 22–23, Prentice-Hall, Englewood Cliffs, N.J.
Kaplan, H. S., K. C. Smith and P. A. Tomlin (1962) *Radiat. Res.* **16**, 98–113.
Koch, A. L., R. J. Doyle and H. E. Kubitschek (1976) *J. Bacteriol.* **126**, 140–146.
Lederberg, J. (1950) *Methods in Medical Research* **3**, 5.
Neidhart, F. C., P. L. Bloch and D. F. Smith (1974) *J. Bacteriol.* **119**, 736–747.
Peak, M. J. (1970) *Photochem. Photobiol.* **12**, 1–8.
Ramabhadran, T. V. and J. Jagger (1976) *Proc. Natl. Acad. Sci. USA* **73**, 59–63.
Robb, F. T., J. H. Hauman and M. J. Peak (1978) *Photochem. Photobiol.* **27**, 465–469.
Russell, A. D. and D. Harries (1967) *Appl. Microbiol.* **15**, 407–410.
Sprott, G. D., W. G. Martin and H. Schneider (1976) *Photochem. Photobiol.* **24**, 21–27.
Swenson, P. A. (1976) In *Photochemical and Photobiological Reviews* (Edited by K. C. Smith), Vol. 1, pp. 269–387. Plenum Press, New York.
Tomlins, R. I., G. L. Vaaler and Z. J. Ordal (1972) *Can. J. Microbiol.* **18**, 1015–1021.
Tuveson, R. W. and R. B. Jonas (1979) *Photochem. Photobiol.* **30**, 667–676.
Tyrrell, R. M. (1976) *Photochem. Photobiol.* **23**, 13–20.
Tyrrell, R. M., S. H. Moss and D. J. G. Davies (1972a) *Mutat. Res.* **16**, 1–12.
Tyrrell, R. M., S. H. Moss and D. J. G. Davies (1972b) *Mutat. Res.* **16**, 345–352.
Tyrrell, R. M. and R. B. Webb (1973) *Mutat. Res.* **19**, 361–364.
Tyrrell, R. M., R. B. Webb and M. S. Brown (1973) *Photochem. Photobiol.* **18**, 249–254.
Van der Schueren, E., D. A. Youngs and K. C. Smith (1974) *Photochem. Photobiol.* **20**, 9–13.
Webb, R. B. (1977) In *Photochemical and Photobiological Reviews* (Edited by K. C. Smith), Vol. 2, pp. 169–261. Plenum Press, New York.
Webb, R. B. and J. R. Lorenz (1970) *Photochem. Photobiol.* **12**, 283–289.
Webb, R. B. and J. R. Lorenz (1972) *J. Bacteriol.* **112**, 649–652.
Webb, R. B. and M. S. Brown (1976) *Photochem. Photobiol.* **24**, 425–432.
Webb, R. B., M. S. Brown and R. M. Tyrrell (1976) *Mutat. Res.* **37**, 163–172.
Webb, R. B. and M. S. Brown (1979) *Photochem. Photobiol.* **29**, 407–409.