Resistance to the Bactericidal Effect of Ultraviolet Radiation Conferred on Enterobacteria by the Colicine Factor colI

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SUMMARY

Strains of Salmonella typhimurium and Escherichia coli which have acquired the colicine factor, colI, are less sensitive to the lethal effects of ultraviolet (u.v.) radiation than the non-colicinogenic parent strain. The dose of u.v. radiation required to kill 50% of the population of S. typhimurium strain LT2 colicinogenic for colicine I was greater by a factor of three than that required to kill 50% of the non-colicinogenic parent. The number of survivors of the non-colicinogenic strain decreased more or less exponentially with dose; the survival curve of the colicinogenic strain had a pronounced 'shoulder'. Experiments with a strain of E. coli K12 carrying lambda prophage indicated that the presence of colI decreased the incidence of phage induction following u.v. irradiation.

INTRODUCTION

Some strains of the Enterobacteriaceae produce colicines which are antibiotics active against some other members of this family, and for this reason are termed 'colicinogenic' (Gratia, 1925; Fredericq, 1957). The ability to produce colicine is attributed to the presence in the colicinogenic strain of a transmissible genetic determinant called a 'colicine factor' regarded as belonging to a group of genetic elements termed 'plasmids' (Lederberg, 1952). Many different colicines are known, the ability to form each one being determined by a different factor.

Non-colicinogenic strains may be made colicinogenic for certain colicines by growth in mixed culture with an appropriate colicinogenic strain (Fredericq, 1954). During culture together, cell conjugation occurs, initiated by the presence of the colicine factor, which is thereby transmitted to the non-colicinogenic strain (Ozeki, Stocker & Smith 1962). Amongst the factors transferred in this way is that responsible for the production of colicine I, that is colicine I factor (henceforth referred to as colI). In Salmonella typhimurium strain LT2 and Escherichia coli strain K12, colI behaves as a sex factor, analogous to the mating factor, F, of E. coli K12, so that under certain conditions not only are colicine factors transferred but also chromosomal genes (Ozeki & Howarth, 1961; Clowes, 1961; Smith & Stocker, 1962).

An attempt was made to increase the number of recombinants arising from colicine-factor-mediated recombination in Salmonella typhimurium, as outlined above, by u.v. irradiation of one or other of the parent strains (see Hayes, 1953); it was then

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found that the parent lines differed considerably in u.v. sensitivity; the strain carrying colI was more resistant than the non-colicinogenic parent.

Colicines are produced spontaneously by only a small fraction of a colicinogenic population and these bacteria are, in consequence, non-viable (Ozeki, Stocker & de Margerie, 1959). Some colicine factors are inducible by u.v. radiation (Fredericq, 1957) but inducibility has not been demonstrated for colI when present in Salmonella typhimurium (Ozeki et al. 1959). Amati (1963) and Monk & Clowes (1964) have, however, reported u.v. induction of colicine I production in Escherichia coli K12. Therefore, one would expect, a priori, the presence of colI to have no effect on u.v. survival or that possibly it might increase sensitivity, if induction did occur.

METHODS

Bacterial strains. Wild-type Salmonella typhimurium LT2 and auxotrophic mutants came from the Department of Genetics, Carnegie Institution, Cold Spring Harbour, New York. Mutant cysD-36 (Clowes, 1958) requires cysteine for growth; athC-5 (Yura, 1956) requires adenine and thiamine for growth. Colicinogenic derivatives of these and other strains are indicated by the addition of the symbol for the colicine factor concerned; for example cysD-36 (colI) is a derivative producing colicine I. The following colicinogenic derivatives, cysD-36 (colI), cysD-36 (ColE1), cysD-36 (colI) (colE2), cysD-36 (colI-17), cysD-36 (colB1) and athC-5 (colI), were from stocks maintained at the Guinness-Lister Unit and have been described by Ozeki, et al. (1962). The source of the colI factor was Shigella sonnei strain 99. Salmonella typhimurium SL427, wild-type LT2 'cured' of a B prophage (Boyd, 1950), maintained at the Guinness-Lister Unit, had been supplied originally by Dr N. Zinder, Rockefeller Institute for Medical Research, New York.

A non-colicinogenic, non-lysogenic subline of Escherichia coli K12 HfrC was obtained from Dr K. W. Fisher, Microbial Genetics Unit, Medical Research Council, Hammersmith Hospital, London. It is an Hfr recombinant of Hfr Hayes cured of lambda (λ) prophage, requiring thiamine for growth, resistant to azide and sensitive to λ phage. Three colicinogenic and/or lysogenic derivatives of HfrC were prepared, (λ) col-, λ- (colI) and (λ) (colI). Lysogeny for lambda phage is indicated by (λ). Salmonella typhimurium strain cysD-36 (colI) was used as the source of colI. Lambda phage for lysogenization was kindly supplied by Dr G. G. Meynell of the Guinness-Lister Unit.

Media. Nutrient broth was made from a tryptic digest of beef. For growth studies involving the use of a nephelometer, cultures were grown in buffered glucose peptone water containing: glucose, 1 g.; peptone, 10 g.; NaCl, 5 g.; sodium glycerophosphate 31 g.; distilled water, 1000 ml.; brought to pH 7.2 by adding N-HCl. Peptone agar, used as a routine medium for viable counts consisted of: peptone (Evans), 20 g.; NaCl, 5 g.; distilled water, 1000 ml.; agar, 13 g.

Culture conditions. Broth cultures were grown in loosely capped 25 ml. bottles ('Universal containers') containing 10 ml. nutrient broth and incubated at 37°, without aeration or shaking. Plate cultures were also incubated at 37°.

Estimation of bacterial growth. Growth curves were plotted from liquid cultures, samples being estimated in a nephelometer (Evans Electroselenium Ltd., Bishop's Stortford, Herts.).
Preparation of colicinogenic derivatives. The methods used for obtaining colicinogenic strains of *Salmonella typhimurium* have been described in detail by Ozeki *et al.* (1962). In the case of *colI*, which is readily transmitted by mixed culture, it consisted of growing together a suitable colicinogenic donor strain and the non-colicinogenic acceptor strain. For preparation of colicinogenic derivatives of wild-type LT2 and SL427, strain *cysD-36 (colI)* was the source of *colI*. A strain of *Escherichia coli*, CL104, was used as the colicine-sensitive indicator strain (Ozeki *et al.* 1962).

*Escherichia coli* is sensitive to colicine I, unlike *Salmonella typhimurium*. Resistant mutants of both the non-lysogenic parental strain HfrC and its derivative lysogenic for λ (see below) were isolated from colonies appearing in colicine-inhibition zones (Fredericq, 1957). Resistant strains were made colicinogenic for colicine I by culture in common with *cysD-36 (coll)*.

Preparation of lysogenic strains of *Escherichia coli* HfrC. Bacteria were infected with phage λ at high multiplicity and survivors tested for lysogeny by u.v. induction.

**Ultraviolet irradiation.** Overnight unaerated broth cultures were centrifuged, washed and resuspended in saline, then usually agitated in the M.S.E. blender for 2 min. at a speed of 12,000 rev./min. to break up clumps and pairs of bacteria. This suspension contained $10^8$-$10^9$ bacteria/ml., depending on the organism. It was usually diluted $10^{-2}$ or $10^{-3}$ in saline for irradiation. A 3 ml. volume of the final suspension was u.v. irradiated in an open Petri dish, on a mechanical rocker, to ensure uniform irradiation. Dose was measured as time of exposure, at a standard distance from the u.v. source. Treated bacteria were plated for viable counts on peptone agar.

Two lamps were used for u.v. irradiation: one was a high-pressure mercury arc giving an output in many regions of the u.v. spectrum; the other was a low-pressure Westinghouse Sterilamp, type no. 782L-30, emitting more than 80% of its radiation in the region of 2537 Å.

**Ultraviolet induction of prophage λ** (Lwoff, Siminovitch & Kjeldgaard, 1950). A saline suspension of λ-lysogenic bacteria containing about $10^7$ bacteria/ml. was plated for viable count and plaque count (Adams, 1959) before and after irradiation. The λ-sensitive strain HfrC was used as an indicator for plaque counts. After overnight incubation bacteria which formed plaques at the time of plating in overlays produced large distinct plaques, distinguishable from the small clearings surrounding most colonies of the lysogenic strain.

To measure the effect of different doses of u.v. radiation on induction, separate 3 ml. samples of the saline suspension were irradiated for increasing periods. To ensure a high percentage of induction in irradiated bacteria, they were incubated in broth for 30 min. before plating for plaque counts (Weigle & Delbrück, 1951).

**RESULTS**

**Effect of colI on the u.v. sensitivity of Salmonella typhimurium LT2**

Typical dose-survival curves, obtained for the pair of strains *cysD-36 col−* and *cysD-36 (colI)*, are given in Fig. 1. The non-colicinogenic strain, *cysD-36 col−*, was killed more or less exponentially, with a slight increase in death-rate at doses greater than 2 min.; the curve of the colicinogenic strain, on the other hand, showed...
a pronounced shoulder. The final slopes of the two curves were about the same. From examination of survival curves the dose of u.v. radiation required to give 50% survival of the non-colicinogenic strain was about 30 sec. and that for the colicinogenic strain about 100 sec. Repeated testing of the two strains, \textit{cysD-36 col}^{-} and \textit{cysD-36 (colI)}, gave reproducible results when the high-pressure mercury arc lamp was used for u.v. irradiation (see Methods). This lamp was used for all experiments to be described, but results were later checked by using a low-pressure Westinghouse Sterilamp, when the same difference in survival of colicinogenic and non-colicinogenic strains was apparent.

In the experiment represented in Fig. 1 possible photo-reactivation was prevented by doing the experiment in a darkened room with only subdued yellow light. However, daylight, when not direct sunlight, had no noticeable effect upon survival and so all subsequent experiments were done under normal laboratory conditions of lighting.

Several possible explanations of the apparent increase in resistance to u.v. radiation, brought about by \textit{colI} agent, were tested experimentally.

(1) That the resistance of the colicinogenic strain was due to clumping of bacteria in the irradiated suspension. As a precaution against clumping, suspensions were routinely agitated in a blender before irradiation, at a speed sufficient to break up all clumps and pairs of bacteria.

(2) That a substance or substances which affected survival after u.v. treatment might be secreted into the medium during growth. In one experiment, an overnight culture of the colicinogenic strain was centrifuged and samples of bacteria resuspended in supernatant fluids of overnight cultures of the colicinogenic and the non-colicinogenic strains where they were left for 1 hr at 37°, before dilution in saline for irradiation. Bacteria of the non-colicinogenic strain were treated in the same way. In a second test, a saline suspension of the colicinogenic strain was u.v. irradiated in the usual way, but before plating for viable counts, the bacteria were diluted in supernatant fluids of overnight cultures of the colicinogenic and of the non-colicinogenic strains. In neither test was the survival of a strain noticeably changed by exposure to the two different supernatants.

(3) That the difference in the sensitivity of the two strains might have been due to differences in sensitivity to the indirect effects of u.v. radiation, for example, to peroxide ions formed in the irradiated medium. This was tested by irradiating bacteria in saline containing 0.1% sodium sulphite, to eliminate free peroxide ions. The difference in the survival curves was the same as obtained by u.v. irradiation in plain saline. Furthermore, when bacteria were u.v. irradiated after being gently spread on the surface of peptone agar plates, when indirect effects of irradiation would be minimized, the difference between the two curves was unchanged, though the rate of killing of both strains was slightly greater than when the same doses of radiation were given to the bacteria in suspension.

(4) The results of Alper & Gillies (1958, 1960) suggest that a slow-growing strain should have a greater chance of survival after u.v. irradiation than a faster growing strain. The growth of cultures of several pairs of strains, non-colicinogenic parent and colicinogenic derivative, were compared by turbidity measurements. The strains tested were \textit{cysD-36 col}^{-} and \textit{cysD-36 (colI)}; \textit{athC-5 col}^{-} and \textit{athC-5 (colI)}; \textit{LT2 (wild-type) col}^{-} and \textit{LT2 (wild-type) (colI)}; \textit{sL427 col}^{-} and \textit{sL427 (colI)}. In no case
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did the growth rate of a pair of strains differ once growth was in the exponential phase. However, in each case the irradiated non-colicinogenic strain showed a longer lag period (about 3 hr after 1 min. dose) than the colicinogenic strain (about 1 hr after 1 min. dose), presumably because there were fewer survivors in the former suspension. A dose of 1 min. was expected to give about 70% survival of the colicinogenic strain and about 15% survival of the non-colicinogenic strain. With increase in dose there was increase in the length of the lag period, more apparent in the case of the non-colicinogenic strain in which the lag period increased to about 5 hr with a 2 min. dose, whereas the lag period of the colicinogenic strain increased only slightly to about 1 hr 30 min. with a 2 min. dose.

![Ultraviolet survival curves of Salmonella typhimurium LT2](Fig. 1)

**Fig. 1.** Ultraviolet survival curves of *Salmonella typhimurium* LT2: ○, strain *cysD-36 col*; ●, the colicinogenic derivative *cysD-36 (colI)*. Bacteria grown overnight in unaerated broth culture, were agitated in an M.S.E. blender for 2 min. at a speed of 12,000 rev./min. to break up clumps and pairs of bacteria, then resuspended in saline at a concentration of about $2 \times 10^8$ bacteria/ml. Separate 3 ml. volumes of this suspension were irradiated at each dose level. Irradiation was carried out in an open Petri dish on a mechanical rocker, using a high pressure mercury arc as u.v. source (see Methods). For viable counts samples were diluted in saline and plated on peptone agar.

![Ultraviolet survival curves](Fig. 2)

**Fig. 2.** Ultraviolet survival curves. ○, *Salmonella typhimurium* LT2 wild-type; ●, the colicinogenic derivative LT2 wild type (*colI*); ○, SM427 (*S. typhimurium* wild-type, cured of a B-phage); ▲, the colicinogenic derivative SL427 (*colI*). Saline suspensions containing about $2 \times 10^8$ bacteria/ml. were irradiated. For other details see legend to Fig. 1.

None of the results outlined gave an explanation for the observed difference in sensitivity to u.v. radiation of a non-colicinogenic strain and a derivative carrying the *colI* factor. The generality of the effect of *colI* in *Salmonella typhimurium* LT2 was established by transmitting *colI* to wild-type LT2 and to SM427 (wild-type *S. typhimurium*, cured of a B phage). These strains and an additional auxotrophic strain, *athC-5 (colI)*, were u.v. irradiated, together with the non-colicinogenic strains from which they were derived. Survival curves of the pairs of strains LT2 (wild-
type) col− and LT2 (wild-type) (colI) and athC-5 col− and athC-5 (colII) were similar to those of the corresponding pair cysD-36 col− and cysD-36 (colII). Strain sl427 was less sensitive to u.v. radiation than wild-type LT2 (Fig. 2), possibly because the B phage carried by wild-type LT2 is to some extent induced by u.v. radiation. However, the u.v. sensitivity of sl427 was significantly decreased by the presence of colI factor (Fig. 2).

The effect of colicine factors, other than colI, on u.v. sensitivity of Salmonella typhimurium, strain LT2

The u.v. sensitivity of strains cysD-36 (col 11–17) and cysD-36 (colB1) were compared with the sensitivity of strain cysD-36 (colI). colB1 resembles colI in being readily transmitted by mixed culture (Ozeki et al. 1962; Stocker, Smith & Ozeki, 1963; Smith, Ozeki & Stocker, 1968); col 11–17, a factor determining production of a colicine different from the ‘standard’ types and common in Salmonella typhimurium strains from Australia (Prof. B. A. D. Stocker, personal communication) is likewise readily transmissible. These factors also conferred relative resistance to u.v. radiation but colB1 to a lesser degree than colI. These results are shown in Fig. 3, together with the results of a further experiment in which strain cysD-36 (colI) was u.v. irradiated, as was a strain colicinogenic for colicine E1, cysD-36 (colE1). The survival curve of the strain cysD-36 (colE1) was similar to that of the non-olicinogenic parent (Figs. 1, 3). As production of colicine E1 is known to be induced by
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u.v. irradiation in S. typhimurium LT2 (Ozeki et al. 1959) one might have expected cysD-36 (colE1) to be more sensitive to u.v. than the non-colicinogenic parent. The absence of such enhanced sensitivity has been observed before (H. Ozeki personal communication). A strain colicinogenic for E2, also u.v. inducible, similarly gave a survival curve resembling that of the non-colicinogenic parent (Fig. 3). The presence of colI in bacteria carrying colE1 increased their survival after u.v. irradiation to about the same extent as when present in non-colicinogenic bacteria. Survival of bacteria carrying both colE2 and colI was slightly lower. Moreover, newly acquired colI is also effective, as was demonstrated by the increased survival of a strain carrying colE1 and colI, of which about half the bacteria had acquired colI during overnight growth in mixed culture with cysD-36 (colI), and the remainder had acquired colI during growth in broth for 2 hr before irradiation.

![Figure 4](Ultraviolet resistance associated with colicinogeny.png)

**Fig. 4.** Influence of colI on the u.v. induction of colE2 in Salmonella typhimurium LT2. Results given in graph A were obtained using strain cysD-36 (colE2) and those in graph B using cysD-36 (colE2) (colI). Both show the percentage of bacteria surviving (colony-formers) and the percentage of bacteria induced to form colicine E2 (lacuna-formers) after u.v. irradiation for 40 sec. and 2 min., 30 sec. (Note: arithmetic scales). Saline suspensions for irradiation contained about $4 \times 10^5$ bacteria/ml. A high-pressure mercury arc was used as a source of u.v. (see Methods). After irradiation samples were diluted in saline and plated on peptone agar for viable counts; a further 0-1 ml. sample was diluted in 5 ml. pre-warmed broth at 37°C and incubated for 90 min. to permit synthesis of colicine by induced bacteria. This suspension was further diluted in broth to yield about $8 \times 10^5$ bacteria/ml. and 0-1 ml. samples plated in streptomycin soft-agar, seeded with about the indicator bacteria, on peptone agar plates. The indicator strain used was a colicine-sensitive, streptomycin-resistant derivative of Escherichia coli, strain cl104.

**Influence of colI on the ultraviolet induction of colE2**

Ozeki et al. (1959) in an experiment on an LT2 line carrying colE2, found that after u.v. irradiation giving about 30% survival, more than half the bacteria, that is the majority of those which did not survive, were induced to form colicine E2. Colicine
produced by a single bacterium formed a clear spot or 'lacuna' in the lawn of a colicine-sensitive indicator strain. At the same dose of u.v. irradiation, the survival of a strain carrying colI is about 80%, much higher than a strain not carrying this factor. As colicine production is a lethal process (Ozeki et al. 1959) it would therefore be expected that increased survival caused by the presence of colI in bacteria also carrying colE2 would be accompanied by a decrease in the proportion of bacteria induced to form colicine E2—that is of bacteria forming lacunae. This prediction

![Fig. 5. Ultraviolet-survival curves of non-lysogenic, non-colicinogenic strain Escherichia coli K12 HfrC and its lysogenic and/or colicinogenic derivatives: ○, strain HfrC λ− col−; ●, λ− (colI); △, (λ) col−; ▲, (λ) (colI). Lysogeny for λ phage is denoted by (λ) and colicinogeny for colicine I by (colI); absence of the two characters is indicated by λ− and col− respectively. Saline suspensions for irradiation contained about 6 × 10^7 bacteria/ml. For further details see legend to Fig. 1.](image-url)

was tested using the method of Ozeki et al (1959). Strains cysD-36 (colE2) and cysD-36 (colE2) (colI) were u.v. irradiated for 30 sec. and for 2 min. 30 sec., doses expected to give about 30% survival for the strain carrying colE2 alone and that carrying colE2 and colI. After u.v. irradiation samples were plated for viable counts; after 90 min. incubation in broth, to permit synthesis of colicine by induced bacteria, lacuna counts were made by plating bacteria in a streptomycin soft-agar layer seeded with a streptomycin-resistant colicine-sensitive indicator strain. The lacunae formed after incubation for 8 hr each represented a single bacterium which had synthesized colicine E2. The proportion of survivors (colony-formers) and of induced bacteria (lacuna-formers) are plotted, on arithmetic scales, in Fig. 4. The presence of colI increased the survival of the bacteria irradiated for
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40 sec. from 26\% to 75\%; and, as predicted, the proportion of lacuna-formers at this dose was correspondingly depressed, from 52\% to 22\%. Survival of the strain carrying colE2 and colI at 2 min. 40 sec., 7\%, was lower than the anticipated value, 80\%. Repeated tests of the u.v. sensitivity of this strain showed it to be slightly more sensitive than a strain carrying colI alone; and this was most apparent when dilute suspensions were irradiated.

Fig. 6. Influence of colI on the u.v. induction of \(\lambda\) phage in Escherichia coli K12 HfrC. Results given in graph A were obtained using a lysogenic, non-colicinogenic derivative (\(\lambda\) col\(^-\)) and those in graph B using a lysogenic colicinogenic derivative (\(\lambda\) (colI)). Both graphs show the percentage of bacteria surviving (colony-formers) and the percentage of bacteria induced to form phage (plaque-formers) with increasing doses of u.v. (Note: arithmetic scales.) Saline suspensions for irradiation contained about \(10^7\) bacteria/ml. A high-pressure mercury arc was used as a u.v. source (see Methods). After irradiation samples were diluted and plated for viable counts on peptone agar; a further 0.1 ml. sample was diluted in 9.9 ml. of pre-warmed broth at 37\° and incubated for 30 min. to allow synthesis of phage in induced bacteria. This suspension was then further diluted to yield about 1000 bacteria/ml. and 0.1 ml. samples plated in a soft-agar layer, seeded with about \(5 \times 10^7\) indicator bacteria, on peptone agar. The indicator strain was a derivative of strain HfrC, resistant to colicine I.

**Influence of colI on ultraviolet induction of prophage in Escherichia coli K12**

As colI reduced the u.v. induction of colE2 its effect on the inducible prophage \(\lambda\) of *Escherichia coli* K12 was investigated. The presence of \(\lambda\) prophage in this strain greatly increases its sensitivity to the killing effect of u.v. irradiation, due to phage induction (Lwoff, 1958). Sub-lines HfrC (\(\lambda\) col\(^-\), \(\lambda^-\) (colI) and (\(\lambda\) (colI) were prepared from a stock of strain HfrC, which is normally non-lysogenic for prophage \(\lambda\). For each of these four strains the survival curve showed a pronounced shoulder (Fig. 5). As expected, the survival of the lysogenic non-colicinogenic strain (\(\lambda\) col\(^-\)) was lower at all doses than that of its non-lysogenic non-colicinogenic parent, \(\lambda^-\) col\(^-\). Furthermore, the presence of colI in the lysogenic strain (\(\lambda\) (colI)
resulted in increased u.v. resistance to about the same extent as it did in the non-lysogenic strain λ⁻ (colI). Thus colI confers some protection against u.v. killing in E. coli K12, both when it is non-lysogenic and when it carries the inducible prophage λ. Since the completion of this work Monk & Devoret (1964) have reported similar increased resistance to u.v. irradiation of non-lysogenic strains of E. coli K12 carrying colI. To see whether the presence of colI in the lysogenic strain would decrease the proportion of bacteria induced to form phage an experiment was made similar to that described in the previous section using the two strains HfrC (λ) col⁻ and (λ) (colI). Ultraviolet irradiated samples were incubated in broth for 30 min. to permit phage synthesis in induced bacteria and were then plated in soft-agar layers seeded with a phage-sensitive colicine-resistant indicator strain. The results (Fig. 6; note that the scales are arithmetic) showed that the lysogenic strain carrying colI gave a higher proportion of survivors at all doses tested, and that the proportion of bacteria induced to phage production by a given dose of u.v. was reduced by the presence of colI; e.g. from 47% to 9% in the 90 sec. samples. On comparison of the curves it is also apparent that for some u.v. doses bacteria which would have formed plaques in the absence of colI were recovered as colony-formers in its presence; e.g. in the 90 sec. sample 47% of the non-colicinogenic lysogenic bacteria formed plaques, but 73% of the colicinogenic lysogenic bacteria survived as colony-formers. Thus the presence of colI partly prevented the lethal inducing effect of u.v. irradiation on bacteria carrying prophage λ. However, comparison of the survival curves of the two colicinogenic strains shows that, even in the presence of colI, the lysogenic strain is much more u.v. sensitive than the non-lysogenic strain—that is, the protection conferred by colI against lethal induction of prophage λ is incomplete.

DISCUSSION

One way in which the colI factor might affect radiation sensitivity would be by increase in the chain length of bacteria, which would cause a shoulder on the survival curve. However, this possibility is not supported by direct microscopic examination which reveals no obvious differences in morphology between colicinogenic and non-colicinogenic bacteria. Secondly, it is possible that the colI factor increases the ploidy of the bacteria; this again would be expected to cause a shoulder on a survival curve. Thirdly, one might suppose that colI increases, in some way, the proportion of bacteria with a pool of RNA and protein precursors adequate to allow these bacteria to withstand lethal damage (Hill, 1963). Gillies & Alper (1960) found that cultures in the stationary phase of growth, where a large intracellular pool of precursors would be expected, gave a survival curve with a pronounced shoulder. It is also possible that colI factor of Shigella sonnei strain v9 carries, in addition to the structural gene for colicine production and the gene or genes concerned in its ability to confer maleness, a further gene for decreased u.v. sensitivity, perhaps comparable to the genetic loci controlling u.v. sensitivity in Escherichia coli (Adler & Copeland, 1962; Howard-Flanders, Boyce, Simson & Theriot, 1962; Rösch, Edelman & Cohen, 1963; Greenberg, 1964). Such a gene may be a structural gene for an enzyme responsible for the repair of u.v. damage or alternatively it may cause de-repression of a host gene controlling the production of a reactivating enzyme. Sauerbier (1962) has proposed an enzymic mechanism to explain the host cell
reactivation of u.v. irradiated phage. Furthermore, Rupert (1961) demonstrated that photo-reactivation is an enzymic process. There is no evidence to support any one of these hypotheses rather than another. The following considerations raise a further possibility.

(1) The mechanism of the lethal effect of u.v. irradiation is not well understood, but in at least one instance, that of *Escherichia coli* k12 lysogenic for phage λ, much of the lethal effect is due to induction, since the lysogenic strain is much more sensitive to killing by u.v. irradiation than its non-lysogenic parent. In bacteria carrying defective forms of inducible prophage, irradiation will be expected to cause lethal induction, even though, because of the defect in the genome of the prophage, no plaque-forming units are released. Defective prophages are apparently not uncommon as laboratory mutants and also occur naturally (Lwoff & Siminovitch, 1951; Appleyard, 1954; Jacob & Wollman, 1956). It is, therefore, possible that much of the lethal effect of u.v. irradiation on the apparently non-lysogenic strains we have investigated (*Escherichia coli* k12 HfrC non-lysogenic for phage λ; and *Salmonella typhimurium* lt2 cured of a B phage) results from induction of undetected defective prophages in these strains—or perhaps inducible defective plasmids of some other sort, perhaps colicine factors, although under conditions used for u.v.-survival tests u.v. inducible colicine factors colE1 and colE2 do not appear to increase sensitivity.

(2) The presence of one sort of plasmid in an irradiated bacterium may prevent induction of a plasmid of some other sort which is also present (Hamon, 1959); and experiments described in this paper (Figs. 4–6) show that the presence of colI considerably reduces the induction of prophage λ in *Escherichia coli* k12 and of colE2 in *Salmonella typhimurium*.

(3) It is therefore proposed, as a speculative hypothesis, that the effect of colI in protecting apparently non-lysogenic and non-colicinogenic strains against the lethal effects of ultraviolet irradiation results from its ability to suppress the lethal induction of a defective plasmid in these strains without itself being induced. Nevertheless, colI is inducible in sublines of *Escherichia coli* k12 as shown by Amati (1963) and Monk & Clowes (1964). Experiments have shown that ultraviolet induction of the production of colicine E2 in *Salmonella typhimurium* is partly prevented by colI, but this effect can hardly account for the increased survival of bacteria carrying colI as well as colE2; because, though colE2 is inducible, its presence does not increase the susceptibility of *S. typhimurium* lt2 to the lethal effects of irradiation. The reasons for this are not known; but if, as is hypothesized, strain lt2 carries an inducible defective plasmid then perhaps the presence of colE2 in u.v. irradiated bacteria results in the lethal induction of colE2 instead of the postulated defective plasmid.

The hypothesis that colI protects by suppressing induction of defective plasmids in the strains concerned predicts that, unlike photo-reactivation, it will decrease the lethal but not the mutagenic effect of irradiation. Preliminary experiments have shown that the presence of colI in some auxotrophic mutants of *Salmonella typhimurium* strain lt2 did not result in a decrease of the number of revertants for a given dose of u.v. radiation but an actual increase (Howarth, to be published).
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REFERENCES


