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Ultraviolet-Induced Changes in the Infectivity of *Agrobacterium tumefaciens*

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The infectivity of *Agrobacterium tumefaciens* strain B6 irradiated with short-wavelength ultraviolet light was followed as a function of dose. Previously reported enhancements of B6 infectivity by ultraviolet irradiation, in samples inoculated after 1.75 hr of dark incubation at 27 C, or immediately following irradiation, were found to occur most frequently after losses in cell viability of 60% and of 90% or more, respectively. Changes in colony-forming ability and tumor-initiating ability with increasing dose showed no obvious correlation until the maximal infectivity promotion of samples inoculated immediately after irradiation was reached. Thereafter, both bacterial responses typically decreased in parallel. With low dose rates, infectivity promotions were obtained with less than 10% loss in cell viability. Data for tumor appearance and tumor growth resulting from inoculations with irradiated cultures showed no significant differences from controls, nor did the age of the bacterial culture or age of the host plant influence the response. The infectivity promotion appears to result from an increase in the proportion of viable cells that will subsequently initiate tumors. The characteristics of this ultraviolet infectivity promotion are shown to be most similar to those found in prophage and bacteriocin induction.

The highly virulent strain B6 of *Agrobacterium tumefaciens* frequently exhibits an increased ability to induce tumors on primary pinto bean leaves after exposure to short-wavelength ultraviolet (UV) light (5). At certain UV doses, a greater promotion of infectivity occurs if a dark period at 27 C separates the UV treatment from the time infectivity is tested. This dark effect attains a maximum after 1.75 hr of incubation, and is prevented by exposure to 402-mμ light, indicating a photoreversible effect at the deoxyribonucleic acid (DNA) level. Only a few treatments have been reported to enhance the infectivity of *A. tumefaciens* (9). A full characterization of this UV enhancement thus promised insight into the mechanism of crown-gall tumor initiation, and was especially attractive in view of the important advances occurring in our understanding of the molecular events involved in UV effects on nucleic acids and bacteria (19).

In this paper, the enhancement of *A. tumefaciens* infectivity that occurs immediately after UV irradiation or after incubation in the dark prior to inoculation is shown to vary systematically with UV dose. An examination of several variable parameters of the pinto leaf assay system with UV-promoted cultures indicates that neither the tumor induction process nor the growth characteristics of the tumors are qualitatively altered by this treatment. The result of the UV treatment appears identical to that achieved by inoculating a much higher concentration of bacteria than indicated by viable-cell counts. Since an infectivity promotion may be obtained after irradiations resulting in only small decreases in the number of viable cells, this suggests that prior to irradiation the probability with which individual bacteria situated at a potential tumor site will successfully initiate a tumor is less than one. The increased infectivity resulting when bacteria are irradiated thus appears to be due to an actual increase in the number of bacteria that will subsequently initiate tumors.

MATERIALS AND METHODS

Bacterial cultures. A. tumefaciens (Smith and Town) Conn strain B6 was used throughout these experiments. The growth media and culture conditions have been described (5). Except as noted, the experiments were carried out with 48-hr stationary-phase cultures. Bacteria were irradiated either in the medium in which they had grown (spent broth = SB medium) or in 0.01

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M Na_2HPO_4 (*p*H 7) containing 0.6% NaCl. Bacteria to be irradiated in the latter medium were sedimented by centrifugation at 8,000 × *g* for 30 min at 2 C, and were suspended in the buffered salt solution. The sedimentation-resuspension steps were repeated twice.

UV irradiations. A low-pressure Hanovia mercury vapor lamp (model 88A-45) with 94% (10.4 w) of its output at 253.7 mμ, mounted under a hemicylindrical aluminum reflector, served as a UV source. Portions (30 ml) of cultures containing ca. 10^{10} bacteria per milliliter were irradiated in a dish having a diameter of 112 mm. The bacterial suspensions were agitated with a magnetic stirrer during the irradiation in a dark room with only the mercury vapor lamp as a source of light. Three distances between the lamp and surface of the irradiated culture were used, depending on the nature of the experiments. They provided dose rates of 67, 35, and 14 ergs mm^{-2} sec^{-1} at the culture surface, as measured by a YSI model 65 radiometer. The highest dose rate was employed in irradiations of bacteria in SB medium and the intermediate dose rate for irradiations of bacteria in buffered salt solution, since it provided a similar rate of decrease in viable-cell count to that obtained under the former conditions. The lowest dose rate was selected for critical examination of portions of the UV dose-response curves of *A. tumefaciens* irradiated in SB medium.

Bacterial viability and infectivity determinations. During irradiation, samples of 1 or 2 ml were removed at various times from the cultures and placed in tubes held in crushed ice. The samples were then diluted with cold sterile distilled water to an appropriate concentration for the pinto leaf bioassay of crown-gall tumor initiation. After inoculation on pinto leaves, samples kept in the dark in an ice bath were serially diluted in sterile distilled water, and 0.1-ml amounts of several dilutions were plated in triplicate. The viability and infectivity of strain B6 are unaffected by these dilution conditions. To simulate more closely the conditions of the bacteria inoculated on leaves, the plates were maintained under fluorescent room light until counted. During the dark interval of 1.5 to 2 hr which separated the time of UV irradiation and inoculation on pinto bean leaves in some experiments, the irradiated samples and controls were shaken in the dark at 27 C (5).

Infectivities were determined by use of 12 to 16 primary pinto bean leaves per sample in the quantitative bioassay described for this host (13). The term "specific infectivity" (SI) is employed for comparing the tumor initiating ability (TIA) of irradiated and control samples of bacterial cultures on a viable-cell basis. The greater the SI of a sample was, the fewer viable bacteria were required per tumor initiated (5, 13).

RESULTS

Bacterial viability and tumor-initiating ability (TIA) after UV irradiation. Figure 1 shows the response of a stationary-phase *A. tumefaciens* culture in terms of cell viability and TIA to increasing doses of UV irradiation. After a lag, cell viability decreased in an exponential fashion, typically extrapolating back to the ordinate at a value suggesting loss of viability to be a three "hit" event. The mean lethal dose (dose resulting in 37% survival) determined from 18 cultures irradiated in buffered salt solution at a dose rate of 35 ergs mm^{-2} sec^{-1} was 3.2 × 10^3 ergs mm^{-2}. Individual cultures varied in their sensitivity from 1.5 × 10^3 to 4.2 × 10^3 ergs mm^{-2}. *A. tumefaciens*, therefore, is relatively resistant to UV irradiation in comparison to other bacteria (8), and this, plus the distinct shoulder in the UV survival curve, suggests that it possesses an efficient system for the repair of UV damage (3).

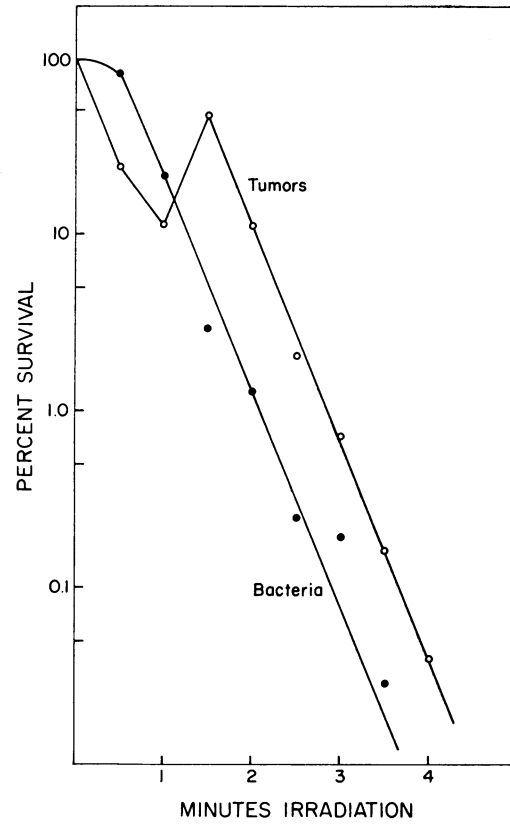

FIG. 1. *Survival of Agrobacterium tumefaciens measured by cell viability and tumor-initiating ability as a function of UV dose (dose rate: 35 ergs mm^{-2} sec^{-1}). Symbols: ●, bacterial viability; ○, tumor-initiating ability. A 48-hr stationary-phase culture of strain B6 was washed three times with buffered salt solution and irradiated in this solution at an initial concentration of 9.2 × 10^9 viable cells per milliliter. The tumor curve is the product of the number of tumors initiated by each sample on 12 pinto bean leaves times the dilution. The actual number of tumors obtained per 12 leaves for the samples from 0 to 3.5 min of irradiation varied between 578 and 29, all in the linear portion of the assay.*

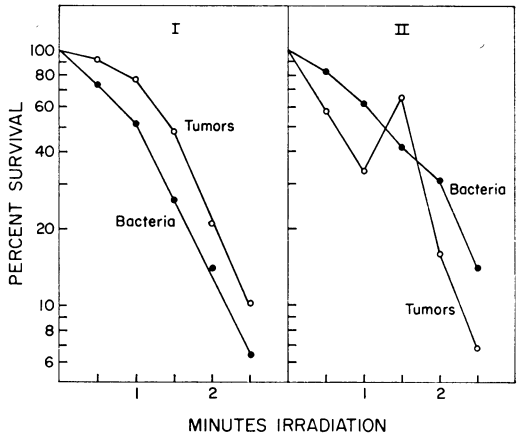

FIG. 2. *Representative patterns of cell viability and tumor-initiating ability survival as a function of UV dose obtained from 13 cultures of varying age. Dose rate and washing procedures were as described in Fig. 1, except that the final bacterial suspensions of each culture to be irradiated were brought to a Klett value of 500. The age of the cultures in hours was as follows: 14, 16.5, 20, 20.5, 24, 24, 31, 33.5, 36.5, 40, 48, and 48, representing log through late stationary phases of growth. Symbols: ●, bacterial viability; ○, tumor-initiating ability.*

If the TIA of the culture were strictly a function of the number of viable cells present, then no significant differences should be observed in the response of these two parameters to UV. The TIA of the culture (Fig. 1), after an initial decrease, however, rose to about half the control tumor level and only then fell off in parallel with cell viability. The specific infectivity (SI) of the culture was nearly 10-fold greater once this latter point was reached. Increases in SI of this magnitude after UV irradiation were about the maximum observed and were obtained in only about 10% of the experiments. The increases in SI were more typically about two- to fourfold, as shown in Fig. 3 and 4 after about 70% loss in viability. The point in the dose-survival curve at which the tumor curve rose above the viability curve was also variable. There is a suggestion in the data that, the greater the UV resistance of the culture is, the more cell viability must be reduced before this rise in TIA is observed. Similar curves and variations have been obtained by irradiating cultures of *A. tumefaciens* strain B6 in SB medium and with other virulent strains of this bacterium.

In an effort to provide some order in these results, 13 cultures of strain B6, varying in age from 14 to 48 hr (log to late stationary phase), were irradiated, and their TIA and cell viability was determined after various UV doses. The two basic patterns of response obtained are shown in Fig. 2. Three of the irradiated cultures show the type I pattern, with TIA showing a greater resistance to UV than cell viability at all UV doses. The type II pattern was also obtained with three cultures and, with some variation, in the remaining seven cultures. The variations consisted of the occurrence of the peak in TIA at 1 or 2 min of irradiation instead of 1.5 min (two cultures), of a TIA peak and curve that failed to rise above the cell viability curve (three cultures), and of a TIA curve that went above and stayed above the cell viability curve after 1.5 or 2 min of irradiation (two cultures). When the TIA patterns for these 13 cultures, however, were considered in sequence according to culture age, there was no obvious trend as to the type of patterns obtained with time.

Figure 3 presents a summary of results from 43 separate experiments relating the effect of UV irradiation as measured by loss of cell viability to the specific infectivity of irradiated cultures measured immediately or after incubation for 1.5 to 2 hr in the dark. The SI curve of samples inoculated immediately after irradiation shows little significant change from the control until UV doses sufficient to reduce cell survival 60% or more have been received. With further increase in dose, larger infectivity promotions were obtained, and the frequency with which individual cultures exhibited a promotion was greater, i.e., after 90% loss of viability. Portions of the cultures receiving the dark treatment after irradiation, however, began to show the infectivity promotion after only a 30% loss of viability, with the maximal promotions occurring at 60 to 70% loss in viability. With greater UV doses, less promotion was observed after dark treatment, and, in the range of 0.1 to 1% survival, no significant promotion was obtained. The rise in this curve in the 0.01 to 0.1% survival range rests on too few data to be regarded as typical, but the high infectivity of cultures immediately after irradiation to this survival level suggests that the dark period may be too short to permit the infectivity to return to the control level. We have shown that the maximal SI obtained in the dark after UV treatment is not stable, and returns to the control level after 1 to 1.5 hr of further incubation in the dark (5). In experiments in which three- to fourfold increases in SI were obtained immediately after irradiation, a 1.5-hr incubation in the dark at 27 C also resulted in a return to the control level of infectivity (G. T. Heberlein, Ph.D. Thesis, Northwestern Univ., Evanston, Ill., 1966). The UV promotion of SI is thus transient in both instances, lending support to the above interpretation.

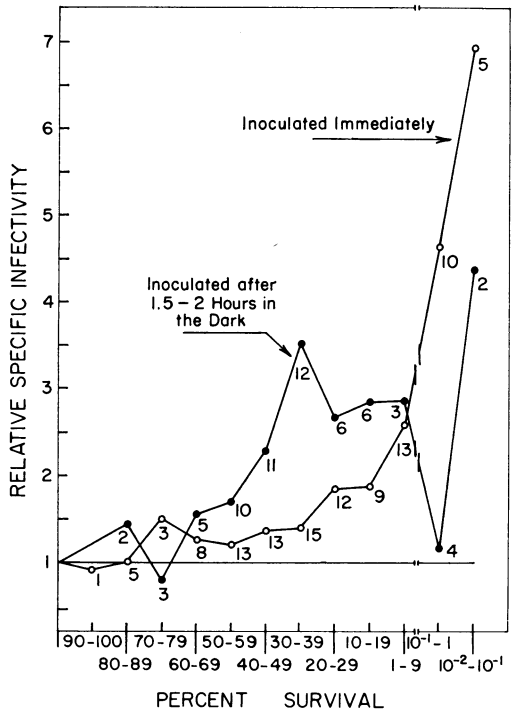

FIG. 3. *Relation between loss of cell viability after UV irradiation and the specific infectivity of the culture as compared with nonirradiated, control portions of the culture. Data from 43 experiments, in which the bacteria were irradiated in buffered salt solution as described in Fig. 1 or in SB medium at a dose rate of 67 ergs mm^{-2} sec^{-1}. Symbols: ○, mean relative specific infectivity of irradiated samples inoculated immediately after irradiation; ●, mean relative specific infectivity of samples held in the dark for 1.5 to 2 hr postirradiation at 27 C. Infectivity data were pooled for each 10% loss of viability. The specific infectivity of the control culture in each experiment was set equal to 1. The numerals beside each point indicate the number of determinations contributing to the mean value. Standard errors of the mean for the points at 30 to 39%, 1 to 9%, and 0.1 to 1% loss of viability were respectively 1.4 ± 0.25, 2.5 ± 0.72, and 4.7 ± 1.0 for the samples inoculated immediately and 3.5 ± 0.7, 2.9 ± 1.2, and 1.17 ± 0.47 for the samples inoculated after a dark period.*

To define this abrupt change in the infectivity of irradiated cultures with greater precision, experiments were carried out in which the UV dose rate was reduced by about 80% to allow convenient removal of more samples per unit dose. In early experiments, a promotion of infectivity was observed following doses of UV light that produced little or no loss in viability; this increase was thought to correspond with the promotion described above but to occur at a lower UV dose because of the changed conditions of irradiation. However, later experiments in which extended irradiation times at this lower dose rate were studied (Fig. 4) showed that there are two regions of UV promotion, since the promotion beyond 60% loss of viability was still present. The first promotion obtained under low dose rate conditions was maximal at 4 to 8 min of irradiation in individual experiments and may be as great as three- to fourfold, despite an insignificant change in the number of viable cells. It was also obtained when washed cells were irradiated in buffered salt solution. After this initial infectivity rise, continued irradiation reduced the TIA of the culture to the control level, until the second rise occurred after a 60% loss in cell viability. With further irradiation, TIA and cell viability appear to decrease logarithmically with increasing dose, as observed in Fig. 1, with the SI of the irradiated sample remaining about two- to threefold greater than the unirradiated control. The first UV promotion was thus initiated and lost by the time a 30% decrease in cell viability had occurred.

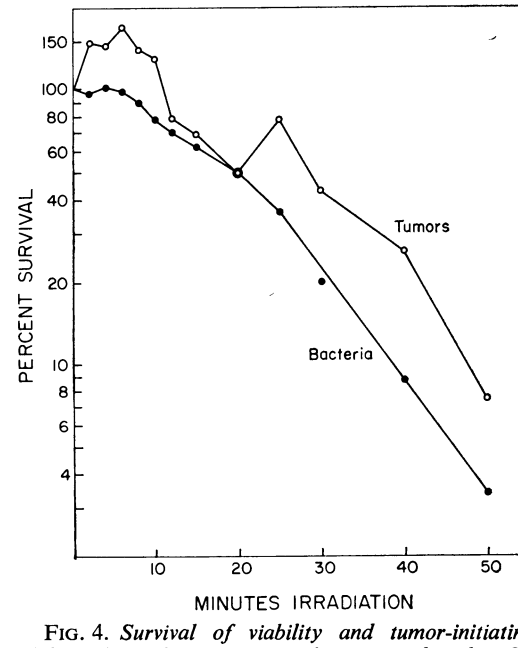

FIG. 4. *Survival of viability and tumor-initiating ability of Agrobacterium tumefaciens irradiated in SB medium as a function of time of irradiation at a dose rate of 14 ergs mm^{-2} sec^{-1}. The two curves are a mean of three experiments except for the tumor data at 0 through 15 min of irradiation, which is a mean of five experiments. In two of the experiments extending over the full 50-min period of irradiation, the culture was set in an ice bath during the irradiation. No significant differences between these experiments and those at room temperature were noted. Symbols: ●, mean cell viability; ○, mean tumor-initiating ability*

The slight dip in the cell viability curve in Fig. 4 between 8 and 20 min suggests that at least two physiological classes of cells distinguishable by their UV sensitivity may occur in these cultures. The physiological state of *Escherichia coli* at the time of UV irradiation has been shown to affect markedly its UV sensitivity (3). This kind of variation could account for the two infectivity promotions, as well as the variability in degree and dose at which the infectivity promotion was obtained in the experiments at higher dose rates. Those cultures exhibiting a 10-fold promotion after a UV-induced decrease in viability of about 70% may have the least heterogeneity in their UV sensitivity, the greater portion of the culture responding only at this dose level. The cell survival curves for type I and type II responses (Fig. 2) show that a considerable difference in UV sensitivity exists in B6 cultures of varying age, and the fact that they are associated with different TIA responses lends support to the above argument.

Nature of the UV enhancement of TIA. Factors such as the physiological age of the *A. tumefaciens* culture or the age of the primary pinto bean leaf have been shown to result in systematic changes in specific infectivity of several-fold (12, 13). Though the mechanism of these changes is unknown, they offered possible routes through which UV irradiation might be exerting its effect on TIA. Also, qualitative changes, such as an enhanced ability to initiate tumors at particular kinds of wounds (11, 14), or to initiate tumors having enhanced growth rates, could account for the UV promotion. These possibilities have been systematically examined.

A comparison of the infectivity of irradiated and nonirradiated portions of a single B6 culture on plants of varying age is shown in Table 1. The specific infectivity of the irradiated sample shows a 4.4-fold promotion. The ratio between the TIA of the control and irradiated samples remained fairly constant on plants varying from 6 to 11 days in age despite a 10-fold drop in sensitivity of old versus young leaves. Thus, the phenomena responsible for the changes in leaf sensitivity are not involved in the increased capacity of UV-irradiated bacteria to initiate tumors.

In a similar fashion, the specific infectivity of bacterial cultures decreased some fivefold between early log and stationary phases of growth (13). Early log-phase cultures, however, may also be promoted in their infectivity by UV irradiation (G. T. Heberlein, Ph.D. Thesis). The factors responsible for the alterations in the infectivity of *A. tumefaciens* cultures of different ages, therefore, appear unrelated to the UV-induced enhancement of infectivity.

TABLE 1. *Infectivity of control and UV-irradiated suspensions of Agrobacterium tumefaciens on pinto bean leaves*

Plant age (days from planting)	Tumors per leaf[a]		Irradiated control
	Control[b]	UV irradiated[b]	
6	43	79	1.8
7	42	75	1.8
8	15	21	1.4
9	8	10	1.25
10	3	7	2.3
11	5	10	2.0

[a] Sixteen leaves used for each determination. Per cent specific infectivity on day 7 plants: control = 100; UV-irradiated = 441.

[b] Stationary-phase culture containing 1.29×10^{10} viable cells per milliliter irradiated to a 20% survival level and inoculated immediately. Control inoculated at a 3×10^{-2} dilution (3.9×10^{8} viable cells per milliliter) and UV-irradiated sample inoculated at a 6×10^{-2} dilution (1.56×10^{8} viable cells per milliliter).

The rate of tumor appearance and the growth rate of tumors on leaves inoculated with UV-irradiated and control samples from a single culture are shown in Tables 2 and 3, respectively.

TABLE 2. *Rate of tumor appearance on pinto bean leaves inoculated with control and UV-irradiated suspensions of Agrobacterium tumefaciens*

Days from inoculation	Control[a]		UV irradiated[a]	
	Tumors/leaf[b]	Per cent of maximum	Tumors/leaf[b]	Per cent of maximum
2	11	31	26	41
3	17	47	32	50
4	23	64	43	68
5	27	75	53	83
6	32	89	59	93
7	34	94	61	96
8	35	97	63	99
9	35.6	99	63.5	100
10	36	100	63.5	100
11	36	100	63.5	100

[a] Stationary phase culture containing 5×10^{9} viable cells per milliliter irradiated to a 26% survival level and incubated for 1.75 hr in the dark along with a nonirradiated control. Per cent specific infectivity at day 11: control = 100; UV-irradiated = 410.

[b] Control inoculated at a 3×10^{-2} dilution (1.5×10^{8} viable cells per milliliter) and UV-irradiated sample at a 5×10^{-2} dilution (6.5×10^{7} viable cells per milliliter). Sixteen leaves used per sample.

TABLE 3. *Tumor growth on pinto bean leaves inoculated with control and UV-irradiated suspensions of Agrobacterium tumefaciens*

Days after inoculation	Control[a]		UV irradiated[a]	
	Tumor diam[b]	Per cent of day 14 value	Tumor diam[b]	Per cent of day 14 value
3	9.5	30.6	12.3	35.2
4	14.3	49	16.3	47
5	18.6	60	20	57
6	22	71	24.6	70
7	25	81	28	80
8	27	87	30.6	87.5
9	29	93.5	32.4	92.5
10	30	97	34.2	97.5
11	30.5	99	34.5	98.5
14	31	100	35	100

[a] Data from the experiment described in Table 2.
[b] Mean of 20 tumors: control tumors from five leaves on three plants; UV-irradiated from five leaves on four plants.

Despite the fourfold specific infectivity promotion obtained after UV irradiation, no significant differences in either parameter have been noted. The irradiated bacteria, therefore, initiate tumors over the same spectrum of wounds in about the same relative frequency as the nonirradiated control, and the tumors initiated have similar growth rates. Furthermore, the rate of tumor appearance data for control and irradiated samples when graphed extrapolate to zero tumors at the same point. Qualitatively, then, the sites of tumor initiation and the growth characteristics of the tumors are not obviously changed when UV-promoted bacteria are used to initiate tumors.

DISCUSSION

These experiments in which colony-forming capacity (viability) and TIA of *A. tumefaciens* are compared as a function of UV dose may be viewed as equivalent to plating irradiated bacteria on two different growth media. Bacterial UV survival curves have been shown to vary, depending upon the medium employed to assay viability (1). If this were the only differential factor operating in our experiments, however, the curves for cell viability and TIA should bear a fairly constant relation in individual experiments. A review of Fig. 1–4, shows that this is rarely the case. In most experiments, infectivity is not obviously correlated with viability until viability has been reduced about 70% or more. The change in infectivity is dependent, therefore, on an event or events initiated by some minimal amount of UV damage. Once this point has been attained, however, TIA decreases in parallel with cell viability, indicating that, in this portion of the dose-survival curve, infectivity changes solely as a function of the number of viable cells. These results re-emphasize the necessity noted earlier of a viable bacterial cell for tumor initiation (12, 13), and further indicate that viability per se of a virulent strain is only one of the requirements for TIA.

A preferential killing of noninfectious cells in the initial portion of the UV dose-survival curve fails to provide a satisfactory explanation of the UV enhancement of *A. tumefaciens* infectivity, for the following reasons: (i) the shape of the TIA curves shows an abrupt change rather than the gradual promotion with UV dose to be expected if preferential killing were the decisive factor; (ii) the number of tumors initiated by samples given UV doses sufficient to reduce viability 50 to 70% are often greater than the number of tumors initiated by the unirradiated control at the same dilution; and (iii) at low UV doses, two- to fourfold promotions of TIA may be obtained in the absence of a significant loss of cell viability.

An investigation of possible avenues for the UV-induced infectivity change suggested from earlier studies of natural parameters of the bioassay which affect TIA independently of bacterial viability (see Tables 1–3) has given only negative results. The SI promotion following UV irradiation seems fully comparable with the inoculation of two to four times more viable unirradiated cells. Thus, the available evidence indicates that a greater portion of the viable cells in an irradiated culture can initiate tumors than in a control culture. The basis for this increase could take one of the following forms: (i) noninfectious cells may be converted to infectious cells in an all-or-none type of response; (ii) all viable cells may have a similar probability of tumor initiation that is increased on receipt of a nonlethal amount of UV-induced damage; (iii) all viable cells may be potentially infectious but exhibit a varying probability of infection depending on their exact physiological state, those cells with low probability of infection being increased on receipt of nonlethal UV damage. A priori, the latter explanation of these results appears more probable, but as yet there are no data to distinguish among these alternatives.

The enhancement of *A. tumefaciens* infectivity by a dark incubation period following irradiation (5) occurs over a limited UV dose range, and is first observed at lower doses than the enhancement obtained when bacteria are inoculated immediately after irradiation. This UV plus dark

postirradiation promotion results in increases in infectivity comparable to those obtained immediately after irradiation with greater UV doses, and disappears at higher levels. Since this dark promotion is counteracted by blue light (5), which may be presumed to induce light-activated DNA repair enzymes (18), repair of UV damage does not appear to be responsible for these changes. One kind of UV-induced genetic change, mutation fixation, has been reported to increase in the dark after irradiation in the absence of viability changes (22). The changes in TIA in the dark following low doses of UV, therefore, may involve a mechanism similar to that responsible for the fixation of bacterial mutations.

The increased SI values observed when cultures receiving greater UV doses are inoculated immediately after irradiation also find a parallel in UV-induced mutation frequency in bacteria. For many bacterial mutations, the absolute number of mutants rises with UV dose, attains a maximum at about 50 to 90% loss of cell viability, and then declines proportionally with cell viability (6), much as infectivity has been found to vary with UV dose in the experiments reported here. Two other systems, prophage and bacteriocin induction, also show this kind of response with increasing UV dose (15).

Each of these UV-induced changes appears to stem largely from pyrimidine dimers (4, 7, 10, 19), as does the UV-induced inhibition of bacterial DNA synthesis (20). This latter response, however, is generally proportional to UV dose up to a maximal delay of DNA synthesis (2) beyond which further increases in dose only serve to reduce the subsequent rate of synthesis. The relative effectiveness of a fixed number of UV-induced dimers in inducing mutations, prophage or bacteriocins, may be related to the length of DNA synthesis inhibition relative to the efficiency of repair systems (2, 4, 7, 10, 16, 17, 21, 22). The absolute number of lesions necessary for these changes, therefore, varies from organism to organism depending upon factors affecting DNA synthesis or repair systems, or both. The variability in our experiments may be explainable on this basis.

The number of dimers per *A. tumefaciens* cell in a culture receiving one mean lethal dose (dose yielding 37% survival) may be estimated from the data of Setlow and co-workers (19) to be of the order of 7,000 or to involve about 0.01% of the total DNA bases. Most of these lesions, therefore, must be repairable, the infectivity promotion depending upon some number of dimers in a limited range between the number necessary for promotion and the number producing a lethal event. This value would vary, depending upon the amount of repair possible between the time of irradiation and the time at which the potential tumor site, where the bacterium resides after inoculation, is either converted to a tumor site or loses its susceptibility. A comparable model has been proposed by Hill (7) for UV-induced mutations.

Mutation frequencies for individual bacterial genes under optimal conditions may attain levels of about 1 per 10,000 survivors (22). Such a frequency is much too low to account for our infectivity results on the basis of changes in one or a few specific genetic loci. This clearly implicates some common event stemming from randomly occurring UV lesions on the bacterial chromosome in the UV promotion of *A. tumefaciens* infectivity. Bacteria show at least three general responses that result from randomly occurring DNA lesions induced by UV light: inhibition of DNA synthesis (2), prophage induction (15), and the induction of bacteriocins (15). The two latter responses are probably dependent upon the former, i.e., the inhibition of DNA synthesis (7), and in some systems essentially the entire population of viable cells following irradiation may be induced (15). Prophage and bacteriocin induction, therefore, have the characteristics of response to UV dose and frequency of occurrence necessary to account for our results. Inhibition of DNA synthesis may thus provide a common mechanism leading to one or more secondary events, any one of which could be associated with the rise in infectivity we have observed. A critical testing of the possibility of prophage or bacteriocin induction in relation to this phenomenon is clearly in order as a further step in the elucidation of this problem.

A working hypothesis consistent with our findings and the literature on UV-induced modifications of bacteria is as follows. The probability with which a viable *A. tumefaciens* cell properly situated in a leaf wound site will initiate a tumor is less than 1. A large portion of bacteria receiving some threshold amount of UV damage at the chromosomal level insufficient to destroy colony-forming ability show an increased probability of infection as compared with their prior condition. The UV lesions responsible for this infectivity change occur in the bacterial DNA and are similar to those resulting in bacterial mutation. The promotion is not observed until some minimal UV dose has been received, owing to the necessity of accumulating sufficient lesions for promotion in enough bacteria to be detected in the pinto leaf bioassay. The location of these lesions is nonspecific, their effect being exerted

through some common mechanism, stemming from a temporary inhibition of bacterial DNA synthesis, e.g., prophage or bacteriocin induction.

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