the impact glass from Lake Bosumtwi fall convincingly on the same isochron, or a little to the left of it, as would be expected had there been a slight loss of rubidium. There can be little doubt that Ivory Coast tektites were made from rocks 2000 million years old, and that such rocks once lay on the spot now covered by Lake Bosumtwi.

Origin of Tektites

The accumulating geochronologic evidence indicates more and more convincingly that tektites were formed from terrestrial rocks in large meteoritic impacts on the earth. Ivory Coast tektites were formed about 1.3 million years ago, simultaneously with the Bosumtwi crater, and from rocks 2000 million years old. The rocks around the crater are of the same age. Moldavites were formed 14.8 million years ago, simultaneously with the Ries crater, and from rocks now roughly 300 million years old. Crystalline rocks throughout Germany north of the Alps are about 300 million years old. North American tektites were formed 35 million years ago, but no crater is known to be associated with them. Their age, as determined by the strontium isochron method, would be compatible with an origin from Appalachian granites, volcanic rock, or sediments derived from them.

Not much can be said about a parent rock for the Australasian tektites, except that it would be roughly 200 to 400 million years old. The principle of simplicity suggests that one might more profitably look for large concealed craters in that vast region than postulate an extraterrestrial origin for the tektites.

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Radiation-Induced Mutations and Their Repair

Bacteria reduce the mutagenic effects of ultraviolet light by repairing DNA damaged by the radiation.

Evelyn M. Witkin

Ultraviolet light kills bacteria, and it also induces mutations among the survivors. For many years, radiobiologists have suspected that both effects start with photochemical changes in the nucleic acids of the exposed cells (*1*). Recently, they have succeeded in identifying specific photoproducts, formed in the DNA of irradiated bacteria, that contribute to the bactericidal effect of ultraviolet light, and have begun to understand how bacteria sometimes repair potentially lethal radiation damage (for recent reviews, see *2–4*). This article explores the roles played by newly discovered products of ultraviolet irradiation and by the mechanisms whereby such damage is repaired in the induction of mutations.

The first photochemical lesion found in the DNA of irradiated bacteria was the thymine dimer (*5*). Ultraviolet light produces thymine dimers mainly by linking adjacent thymine bases in the same strand of DNA, via carbon-to-carbon bonds. (Normally, of course, the purine and pyrimidine bases in a single strand of DNA are connected only to the sugar-phosphate "backbone," and not to each other.) Other pyrimidine dimers (cytosine-cytosine and cytosine-thymine) are also formed in irradiated DNA, but probably less efficiently than dimers of thymine (*2, 6, 4*). Dimers containing thymine block DNA replication in vitro (*7*) and in vivo (*8*) and are responsible for an important fraction of the lethal effects of low doses of ultraviolet light in some strains of bacteria (*8*).

Pyrimidine dimers are subject to repair in the bacterium *Escherichia coli*. They may be eliminated from the DNA of irradiated cells in one of two known ways. The first requires exposure, after irradiation, to an intense source of visible light (the most effective wavelengths being those around 4000 angstroms), a treatment known to reverse or "photoreactivate" many of the biological effects of ultraviolet light (*9*). In one kind of photoreactivation, pyrim-

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idine dimers are split, *in situ* (*10*), by a light-dependent "photoreactivating enzyme" found in *E. coli* and yeast (*11*). Alternatively, in the dark, pyrimidine dimers are removed from the DNA, apparently by the excision of short, single-stranded segments that include the dimers and a small number of neighboring nucleotides (*12*). Subsequent steps in the dark repair of pyrimidine dimers are not known in detail, but the gap left by excision is presumably filled by "repair synthesis," with the nucleotide sequence opposite the excised segment serving as template. Evidence for a "patching" mechanism following excision has been described (*13*).

These advances in our knowledge of the effects of ultraviolet light on DNA, and of the ways in which cells minimize radiation damage by repair, raise many questions about mutagenesis. Do pyrimidine dimers cause mutations? Are some ultraviolet-induced mutations caused by photoproducts that are demonstrably different from pyrimidine dimers? Does repair of radiation damage reduce the chance of induced mutation, or are most induced mutations caused by "mistakes" in the repair process itself? Is repair of radiation damage equally efficient in all genes? Is there any specificity in the kinds of products of ultraviolet irradiation that cause mutations in different genes? Direct biochemical methods are not very helpful in answering such questions, since an induced mutation is a rare and elusive event. The experiments described here make use, instead, of mutant strains that differ from each other in their ability to effect particular kinds of repair of radiation damage. Comparing mutagenesis in pairs of such strains has yielded some preliminary answers to the questions posed.

Material and Methods

The experiments, except where noted, were done with strains WP2, a tryptophan-requiring derivative of *Escherichia coli* B/r, and $WP2_s$, an ultraviolet-sensitive mutant strain derived from WP2 (*14*) and also known as WP2 (*hcr*$^-$). Methods of growing cultures, preparing them for irradiation, and exposing them to ultraviolet light and photoreactivating light were all as previously described (*15*), unless otherwise specified. In experiments in which ultraviolet light doses of less than 100 ergs per square millimeter were used, the dose rate of the lamp was reduced about 20-fold by masking all but a 2.5-centimeter central section of the bulb in aluminum foil (*16*).

Three kinds of genetic changes were investigated: mutations from streptomycin-sensitivity to streptomycin-resistance; from ability to inability to ferment lactose; and from auxotrophy (growth-factor requirement) to prototrophy (no growth-factor requirement). Streptomycin-resistant mutants were selected as previously described (*17*), except that streptomycin was added to the plates after 6 hours of incubation, and colony counts were made after 3 days. Mutants which were unable to ferment lactose were detected by inspection of colonies on Bacto eosin-methylene blue indicator agar after 2 days of incubation. Only mutants that formed colorless colonies (or sectors of colonies) on these plates and failed to grow when lactose was the sole carbon source were scored as "lactose-negative." Mutations from auxotrophy to prototrophy were selected on "semi-enriched minimal" (SEM) agar (*15*), a minimal agar containing 5 percent nutrient broth. Survival was determined on the same media used to detect the induced mutations, except that streptomycin was omitted from the plates used to assay survival in experiments with streptomycin-resistance. All mutation frequencies are corrected for spontaneous mutations.

Mutations and Excision

Strain WP2, like its parent B/r, is about as resistant to ultraviolet light as any strain of *E. coli*, and possesses, in active form, all genes known to promote repair of radiation damage. Strain $WP2_s$, which arose by mutation from WP2, is about 20 times more sensitive to ultraviolet light and lacks measurable ability to excise thymine-containing dimers from its DNA (*14*). The finding that many strains of *E. coli*, differing widely in their sensitivity to ultraviolet light, all have the same number of thymine-containing dimers produced in their DNA by the same ultraviolet dose (*8*) supports the assumption that the same ultraviolet dose produces the same amount of damage in the two strains and that the only relevant difference between the strains is their excision ability. Thus the sensitivity of strain $WP2_s$ can be ascribed to reduced repair of radiation damage, resulting from the failure of normal excision. A comparison of mutagenesis in WP2 and $WP2_s$ should tell us whether the excision of damaged segments of the DNA affects the induction of mutations. Hill's finding (*14*) that $WP2_s$ is much more susceptible than WP2 to the induction of mutations to tryptophan-independence indicates that the loss of detectable excision ability greatly increases the probability of this kind of mutation.

Figure 1 shows survival and the frequency of induced mutations resulting in independence of tryptophan in strains WP2 and $WP2_s$, after various doses of ultraviolet light. The results confirm those of Hill in that they show that mutations of this kind are produced abundantly in the sensitive strain at doses far below those required to induce comparable numbers in the resistant strain.

Figure 2 shows survival and the frequency of mutations resulting in resistance to streptomycin, in the sensitive and resistant strains, as a function of ultraviolet dose. Strain $WP2_s$ is much more susceptible than WP2 to the induction of mutations to streptomycin-resistance, the frequencies obtained at very low doses actually exceeding those observed at any dose in the resistant strain.

Table 1 shows the frequency of mutations resulting in failure to ferment lactose induced in the sensitive and resistant strains by ultraviolet light. These mutations, too, are produced in strain $WP2_s$ at a dose too low to show a significant mutagenic effect in the resistant strain.

The higher mutation frequencies found in the sensitive strain $WP2_s$, in association with reduced excision capability, indicate that photoproducts of the kind that are potentially excisable can play an essential part in the induction of all three types of mutation. If only nonexcisable photoproducts were involved in mutagenesis, differences in excision ability would be irrelevant, and the frequency of induced mutations should be the same, at a given dose, in the sensitive and resistant strains. Since mutation frequencies are lower when excision is normal, we may conclude also that mutations are less likely to occur as "mistakes" in the course of normal repair than as errors due to the failure or derangement of normal repair itself. If there is no excision at all in the sensitive strain, the mutations observed in $WP2_s$ must be caused, directly

or indirectly, by unexcised photochemical lesions. If the sensitive strain is actually able to excise radiation damage, but only at a rate too low to be detected, it is possible that the mutations in $WP2_s$ are caused by errors specific to abnormal repair following extremely slow excision. In either case, normal excision greatly reduces the probability of mutation. Thus at least 99 percent of the mutations that would otherwise result from ultraviolet irradiation are prevented from occurring, in the resistant strain, by repair processes that include excision. This conclusion depends upon the comparison of mutation frequencies at the same ultraviolet dose in sensitive and resistant strains, and therefore applies only to the very low doses (less than 100 erg/mm^2) at which mutagenesis can be studied in the sensitive strain. Other evidence (*18*), however, suggests that mutation frequencies are greatly reduced by repair of ultraviolet damage at much higher doses, as well.

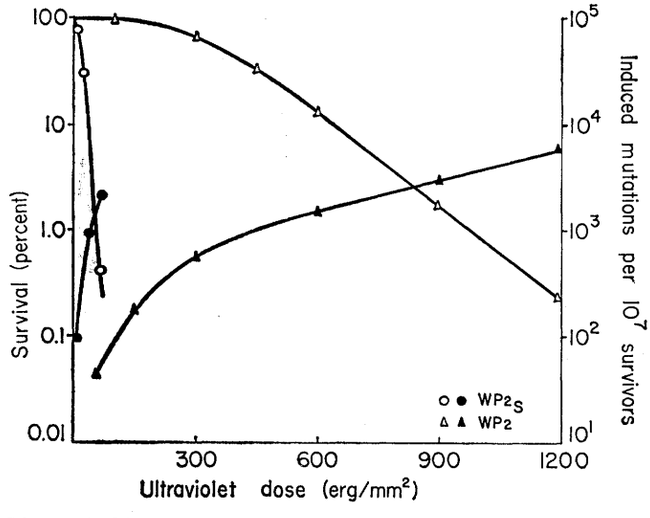
It is important to know whether the loss of detectable excision ability, in the sensitive strain, increases sensitivity to the lethal and mutagenic effects of ultraviolet light equally. If so, the mutation frequencies obtained in the sensitive and resistant strains, at doses having equivalent effects on survival, should be the same. As shown in Figs. 3 and 4, the survival curves of the two strains may be superimposed if appropriate scales of ultraviolet dose are used, the resistant strain requiring about 17 times as much radiation as the sensitive strain to produce the same lethal effect. At doses of ultraviolet light equated for survival, mutations to tryptophan-independence (Fig. 3) are about twice as frequent in the resistant strain (*19*). If the relative sensitivity of the two strains is expressed as the difference in the amount of radiation required to produce equal effects, $WP2_s$ is 17 times as sensitive as WP2 to the lethal effect of ultraviolet light, but only about ten times as sensitive to the induction of such mutations.

A different result is obtained with mutations to streptomycin-resistance, as shown in Fig. 4. In this case, doses equated for survival produce almost ten times as many mutations in the sensitive strain, and thus it appears that susceptibility to the induction of this kind of mutation is increased much more, in strain $WP2_s$, than is sensitivity to the lethal effect. The loss of detectable excision ability is clearly not accompanied by equal increases in the sensitivity to lethal and mutagenic effects, or by equal increases in the sensitivity to the induction of different mutations. Survival is probably influenced by the production and repair of potentially lethal radiation damage anywhere in the DNA, whereas the frequency of a particular kind of mutation is likely to be affected only by the fate of photoproducts produced in a particular gene or small group of genes. Unequally increased sensitivity to killing and to the induction of various mutations, in a strain owing its sensitivity to reduced excision ability, could mean (i) that repair processes involving excision are more efficient in some parts of the DNA than in others, or (ii) that the amount of irreparable damage varies in different regions of the DNA. For example, the lower yield of mutations leading to streptomycin-resistance induced in the resistant strain, by doses equated for survival, could indicate either that repair is much more efficient

Table 1. Frequencies of mutations induced by ultraviolet light that result in inability to ferment lactose in *Escherichia coli* strains WP2 and $WP2_s$.

Treatment after irradiation	Ultraviolet dose (erg/mm^2)	Survival (%)	No. of colonies screened	No. of mutants		No. of induced mutants per 10^8 survivors
				Whole colony	Sectored colony	
			Strain WP2			
None	0	100	26,588	2	0	
None	60	100	32,294	3	0	0
None	1200	0.92	46,312	12	17	540
			Strain $WP2_s$			
None	0	100	24,438	1	1	
None	60	1.2	55,917	15	23	589
PRL*	60	100	48,935	8	28	653

* PRL, photoreactivating light; 10-minute exposure to visible light immediately after irradiation with ultraviolet light.



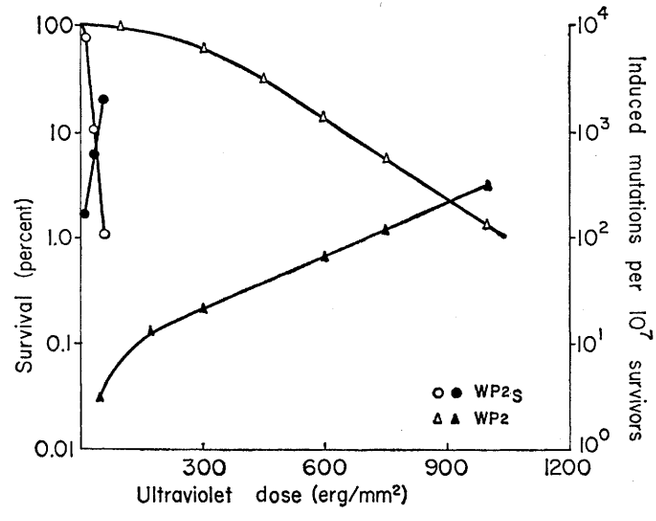


Fig. 1 (left). Survival and frequency of induced mutations that result in independence of tryptophan in *Escherichia coli* strains WP2 and $WP2_s$, after ultraviolet irradiation. Open symbols, survival; closed symbols, mutation frequency. Each point is the average of four experiments. Fig. 2 (right). Survival and frequency of induced mutations that result in resistance to streptomycin in strains WP2 and $WP2_s$, after ultraviolet irradiation. Open symbols, survival; closed symbols, mutation frequency. Each point is the average of four experiments.

in the streptomycin locus than in the DNA as a whole, or that this locus contains an unusually small residue of irreparable ultraviolet damage, or both.

Mutations and Pyrimidine Dimers

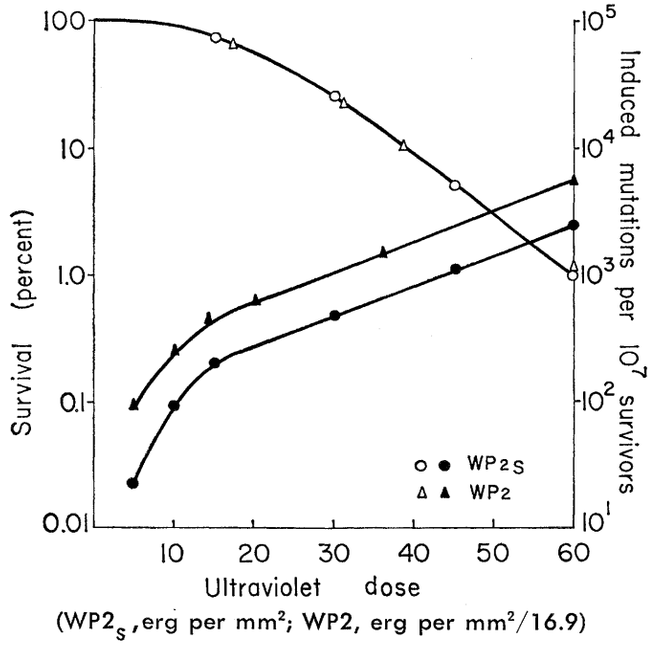
Potentially excisable products of ultraviolet irradiation are involved in the induction of mutations in strain $WP2_s$. Since the specificity of excision is not known, the foregoing experiments do not tell us whether the premutational photoproducts are pyrimidine dimers or some other kinds of ultraviolet damage that may also be excisable. One way to find out is to study the photoreversibility of the mutations induced in the sensitive strain. Photoreversibility alone is not an adequate criterion for the involvement of pyrimidine dimers in an ultraviolet-initiated effect, since, in addition to the light-dependent splitting of pyrimidine dimers by "photoreactivating enzyme," there are indirect mechanisms of photoreversal that do not require this enzyme and that probably do not operate by monomerization of pyrimidine dimers (*20*). Photoreversibility of mutations by the direct, enzymatic mechanism, however, would indicate that pyrimidine dimers are involved in the induction of the mutations, since dimer-splitting appears to be the only direct photoreversal mechanism of which *E. coli* is capable (*4*).

Figures 5 and 6 show that about 90 percent of the mutations to tryptophan-independence and to streptomycin-resistance, respectively, that are induced in the sensitive strain $WP2_s$ are eliminated by exposure to visible light. No significant part of this reversal can be ascribed to indirect effects of the light, since (i) wavelengths known to produce such effects were excluded by a filter (*21*); (ii) no reversal of mutations was obtained if the exposure to visible light *preceded* irradiation with ultraviolet light (a condition that permits indirect photoreversal, but not enzymatic dimer-splitting) (*20*); and (iii) induced mutations resulting in prototrophy or streptomycin-resistance were not reversed by exposure to visible light in another ultraviolet-sensitive strain that lacked the activity of the photoreactivating enzyme and presumably was unable to split pyrimidine dimers (*22*). It seems certain that the photoreversibility of the mutations to streptomycin-resistance and to tryptophan-independence induced in strain $WP2_s$ is of the direct, enzymatic kind in which pyrimidine dimers are split. Pyrimidine dimers, therefore, must participate in the induction of at least 90 percent of these mutations.

In contrast to these results, induced mutations resulting in inability to ferment lactose, as shown in the last two lines of Table 1, are not photoreversible to any significant extent. The frequency of lactose-negative mutants in $WP2_s$ is just as high among cells treated with photoreactivating light as among controls kept in the dark, despite the hundred-fold increase in the number of survivors due to the photoreversal of potentially lethal damage. Products of ultraviolet irradiation that lead to lactose-negative mutations are excisable but are not reversible by visible light. This indicates either that they are not pyrimidine dimers or that, if they are, conditions specific to the loci at which these mutations arise prevent the monomerization of pyrimidine dimers by "photoreactivating enzyme." Nonphotoreversibility of certain mutations resulting in fermentation inability has been reported previously (*23*).

Mutations in Resistant Strains

Is the residual, unrepaired damage that gives rise to mutations in resistant strains qualitatively different from the larger amount of damage that is effectively repaired? Or does it comprise a random sampling of similar damage and merely reflect the limitations of the



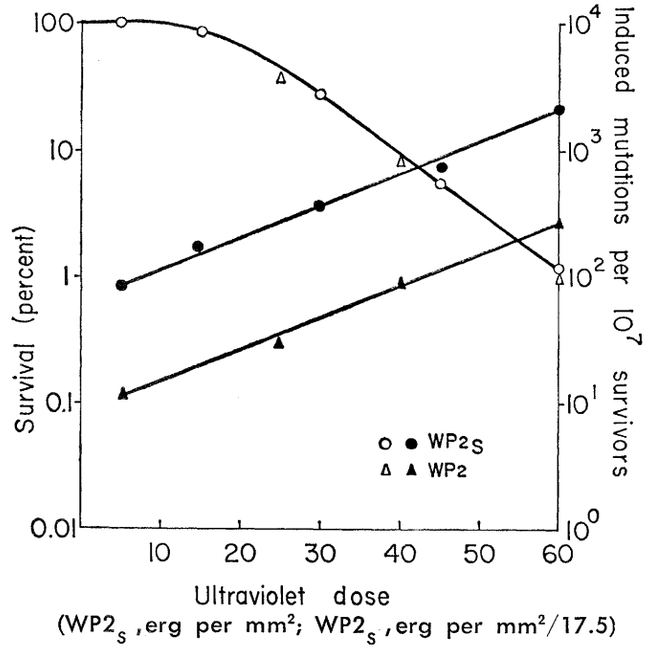


Fig. 3 (left). Frequency of induced mutations that result in independence of tryptophan in strains WP2 and $WP2_s$ after treatment with ultraviolet irradiation at doses resulting in equal survival. Open symbols, survival; closed symbols, mutation frequency. Each point is the average of four experiments. Doses used for strain WP2 were 16.9 times those shown on the abscissa. Fig. 4 (right). Frequency of induced mutations that result in resistance to streptomycin in strains WP2 and $WP2_s$ after treatment with ultraviolet irradiation at doses resulting in equal survival. Open symbols, survival; closed symbols, mutation frequency. Each point is the average of four experiments. Doses used for strain WP2 were 17.5 times those shown on the abscissa.

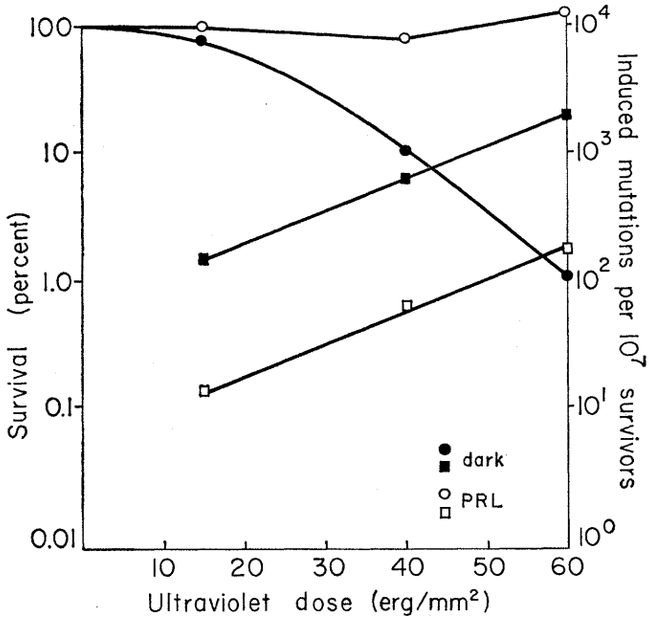
repair systems? Since pyrimidine dimers are involved in the induction of most mutations resulting in tryptophan-independence and streptomycin-resistance in the sensitive strain $WP2_s$, these questions really ask whether the same is true of the mutations induced in the resistant strain.

For ultraviolet-induced mutations to streptomycin-resistance, in the radiation-resistant strain B/r, about 90 percent of the mutations obtained in the dark are eliminated by treatment with light, and this reversal does not occur at all in strains lacking photoreactivating enzyme (*24*). Products of ultraviolet irradiation that are involved in the induction of mutations to streptomycin-resistance, in both sensitive and resistant strains, are subject to direct, enzymatic photoreversal, and may therefore be described as pyrimidine dimers. Both fractions of the premutational damage (the fraction effectively repaired in resistant strains, and the fraction not repaired) include pyrimidine dimers.

No such simple situation exists for mutations to tryptophan-independence induced in strain WP2, however. These mutations belong to a class (broadly described as mutations from auxotrophy to prototrophy) that has been intensively investigated in ultraviolet-resistant derivatives of strain B/r. Although the induction of most of the mutations to tryptophan-independence obtained in the sensitive strain $WP2_s$ (corresponding to those normally repaired in resistant strains) involves pyrimidine dimers, the same kind of mutation produced by higher doses in resistant strains appears to originate from products of ultraviolet irradiation that have unusual properties. These mutations are irreversibly eliminated by a process that has been interpreted as enzymatic dark repair (*18, 25*), if conditions immediately after ultraviolet irradiation are unfavorable for protein synthesis, and mutations are obtained in large numbers only if conditions after irradiation permit active protein synthesis (*26*). Since the same treatments that eliminate mutations to prototrophy (amino acid deprivation, chloramphenicol inhibition) fail to affect survival and the frequency of mutations to streptomycin-resistance (*17*), the premutational photoproducts leading to induced prototrophy must differ, somehow, from those responsible for death and streptomycin-resistance. Not only does the specificity of the conditions promoting its dark repair set this class of mutations apart, but so does its photoreversibility. Mutations to prototrophy are photoreversible in resistant strains (*27*) as well as in sensitive strains. In the sensitive strain $WP2_s$, all this reversibility is accounted for by direct, enzymatic dimer-splitting, as shown above. In resistant strains, on the contrary, induced prototrophy remains fully photoreversible when photoreactivating enzyme and dimer-splitting ability are eliminated by mutation (*24, 28*). Even in strains possessing the ability to split pyrimidine dimers, most of the photoreversal of induced prototrophy can be shown to be of the indirect type, since it can be prevented by treatments applied after the exposure to reactivating light, and hence after any dimer-splitting that may contribute to the photoreversal is completed (*15*). The absence of dimer-splitting as a direct component in their photoreversal, as well as the specificity of their dark repair, has led to the hypothesis (*28*) that the photoproducts responsible for the mutations to prototrophy observed in resistant strains are not pyrimidine dimers (or at least not pyrimidine dimers having normal reparability).

Suppressors and Repair

Not all auxotrophic strains respond to ultraviolet light by producing mutations to prototrophy that have unusual repair properties. Among auxotrophic substrains of *E. coli* B/r, isolated at



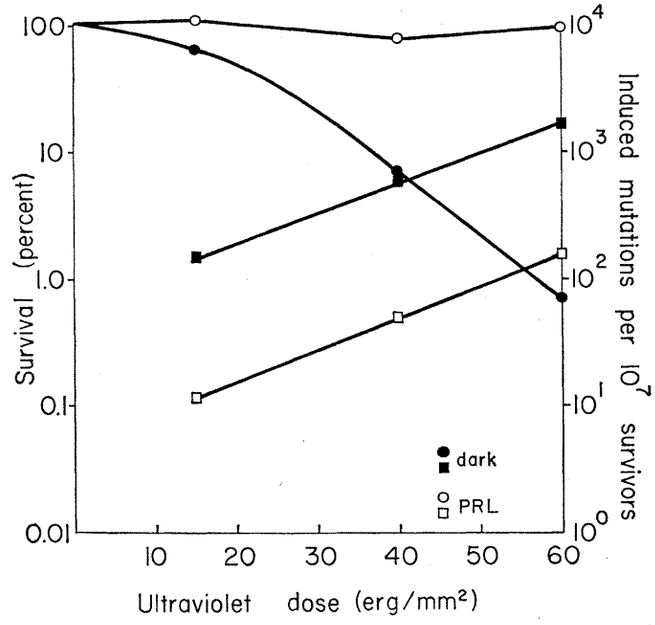


Fig. 5 (left). Reversal by visible light, of potentially lethal damage and induced mutations that result in independence of tryptophan in strain $WP2_s$. Circles, survival; squares, mutation frequency. PRL = 10 minutes' exposure to photoreactivating light immediately after ultraviolet irradiation. Each point is the average of three experiments. Fig. 6 (right). Reversal by visible light, of potentially lethal damage and of induced mutations that result in resistance to streptomycin in strain $WP2_s$. Circles, survival; squares, mutation frequency. PRL = 10 minutes' exposure to photoreactivating light immediately after ultraviolet irradiation. Each point is the average of three experiments.

random, only about 20 to 30 percent give relatively high yields (at least 1 per 10^5 survivors) of induced mutations to prototrophy that can be greatly reduced by omission of amino acids from the plating medium used after irradiation, and that may hence be considered subject to the kind of dark repair that is blocked by protein synthesis. In all auxotrophic strains that do respond in this way, including strain WP2, most of the ultraviolet-induced prototrophs owe their independence of growth factors to suppressor mutations, each capable of correcting a large number of mutational defects scattered around the genetic map (*29*). Suppression of this kind is believed to operate through alterations in the specificity of the genetic code (*30*). The unusual repair properties of the ultraviolet-induced suppressor mutations may reflect singularities associated with genes coding one or more elements of genetic "translation" (activating enzymes, transfer RNA's, ribosomes). The singularity could be primary (unique bases or base sequences leading to unique ultraviolet photoproducts) or secondary (unusual physical state of the DNA at the suppressor loci that affects reparability of radiation damage).

Gorini and Kataja (*31*) have described "conditional streptomycin-dependent" auxotrophs, in which the auxotrophic defect is corrected by streptomycin, and have also shown that the same defect is correctable by suppressor mutations (*32*). Since streptomycin affects ribosomes, thereby altering the specificity of incorporation of amino acids in vitro (*33*), it is likely that genetic suppressors of defects suppressible by streptomycin also change the reading of the code. In a group of 30 conditional streptomycin-dependent auxotrophs tested for their response to ultraviolet light, all but one yielded high frequencies of ultraviolet-induced suppressor mutations that are subject to "amino-acid-sensitive" dark repair (that is, they are obtained only on media enriched with a pool of amino acids). Suppressibility by this kind of mutation is highly correlated with suppressibility by streptomycin among auxotrophic strains selected for their response to streptomycin. These findings support the view that ultraviolet-induced suppressors exhibiting amino-acid-sensitive dark reparability originate in genes that code activating enzymes, transfer RNA's, or ribosomal components, and that their unusual repair properties may be related to singularities at one or more of these loci.

Suppressors and Excision

In resistant strains, suppressor mutations induced by ultraviolet light are obtained in large numbers only if conditions after irradiation favor protein synthesis. If protein synthesis is briefly inhibited after irradiation (for instance, by amino acid deprivation or treatment with chloramphenicol), the potential mutations are irreversibly lost. Actually, only a small fraction of the premutational ultraviolet damage capable of leading to suppressor mutations is subject to this amino-acid-sensitive dark repair. Most of the premutational photoproducts are effectively repaired, in resistant strains, whether the medium contains amino acids or not. This can be inferred from the much higher yield of mutations in the sensitive strain, which lacks detectable excision ability, as well as from the greatly increased mutation frequencies obtained when repair, in resistant strains, is reduced phenotypically by treatment, after irradiation, with such agents as caffeine (*34*) or acriflavine (*18*). Since neither the survival nor the frequency of induced mutations to streptomycin-resistance is affected by deprivation of amino acids after irradiation (*17*), neither seems to depend upon photoproducts that are subject to dark repair only under conditions of inhibited protein synthesis. Such photoproducts, as far as we know, are associated exclusively with the genes in which suppressor mutations originate, where they constitute a small, but demonstrable, fraction of the total premutational ultraviolet damage.

The only dark-repair mechanism known in any detail includes, as an essential step, the excision of radiation damage from the DNA. Does the amino-acid-sensitive dark repair of suppressor mutations also involve excision? Several striking parallels exist between the excision of thymine-containing dimers and the loss of suppressor mutations that occurs when protein synthesis is inhibited after irradiation: both processes show kinetics that are exponential and dose-dependent (*34*, *35*); both require energy (*26*, *4*); both occur in the presence of chloramphenicol (*26*, *4*); and both are blocked by acriflavine (*15*, *35*). The most convincing evidence that the amino-acid-sensitive dark repair of suppressor mutations involves excision comes from mutant strains deliberately selected for their loss of the ability to carry out "mutation frequency decline," as the disappearance of suppressor mutations when protein synthesis is inhibited is sometimes called. These "*mfd*−" mutants produce high yields of induced suppressor mutations even when plating is preceded by a period of amino acid deprivation or chloramphenicol inhibition as shown in Fig. 7, in which an *mfd*− mutant is compared with its *mfd*+ parent strain (*36*).

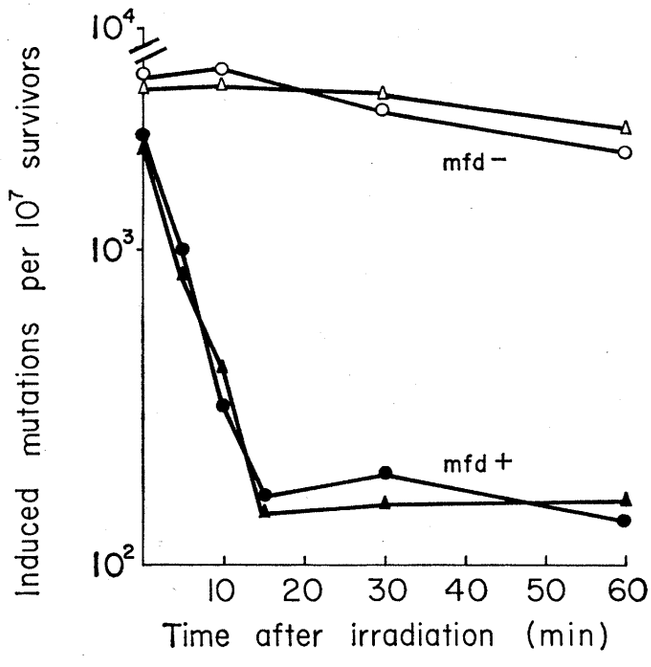
The frequency of induced suppressor mutations ultimately obtained drops rapidly as the *mfd*+ strain is incubated in minimal medium or chloramphenicol, but is reduced only slightly in the *mfd*− mutant. Although the ultraviolet-sensitivity of the *mfd*− strain is the same as that of the resistant strain from which it was derived, the *mfd*− strain excises thymine-containing dimers at a rate markedly lower than normal (*37*). Mutants unable to carry out mutation frequency decline have been isolated repeatedly, and all recover from the inhibition of DNA synthesis following irradiation more slowly than normal. This fact suggests that a reduction in the rate of excision of radiation damage invariably accompanies loss of the ability to effect amino-acid-sensitive dark repair of suppressor mutations. Although other explanations are possible, this association of the two effects in a single mutation is most simply explained by the assumption that excision of thymine-containing dimers and mutation frequency decline are mediated by the same enzyme. Figure 8 shows that strain $WP2_s$, the sensitive strain lacking detectable excision ability, is also unable to carry out mutation frequency decline. In this strain, the frequency of induced suppressor mutations (to tryptophan-independence) is scarcely reduced by conditions that promote mutation frequency decline in the resistant strain WP2, after a dose equivalent with respect to survival. This might also seem to support the hypothesis that excision is an essential step in the amino-acid-sensitive dark repair of suppressors, but the association of the two effects is less meaningful in the sensitive strain than in the resistant *mfd*− mutant. In sensitive strains, the mutations observed arise primarily from damage that is usually repaired, in re-

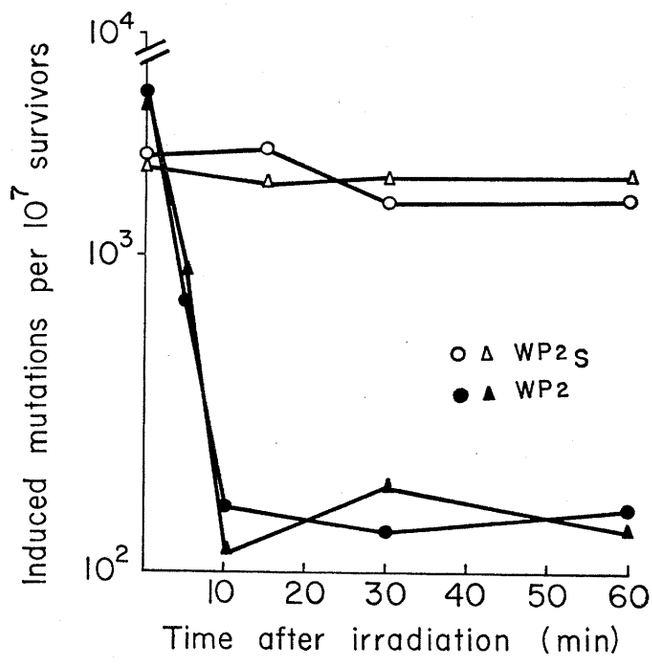
sistant strains, whether amino acids are present or not. This fraction of the damage may simply not be subject to mutation frequency decline. The reduced excision rate observed in the ultraviolet-resistant *mfd*− mutants, however, does support the idea that mutation frequency decline involves the excision of a fraction of the premutational damage, produced in certain suppressor loci, that can be excised only if protein synthesis is inhibited after irradiation.

If the amino-acid-sensitive dark repair of suppressor mutations does include excision, why does it occur only if protein synthesis is inhibited immediately after exposure to ultraviolet light? One possibility is that only under these conditions can the unique photoproducts at the suppressor loci bind excision enzyme. Protein synthesis after irradiation may trigger a change in the state of the DNA as a whole, or of the suppressor loci specifically, such that the ability of the unique photoproducts to compete successfully with pyrimidine dimers for available excision enzyme is reduced. If the suppressor genes code elements of translation, one consequence of inhibiting protein synthesis might be the repression of the suppressor loci. It is possible that the recognition of radiation damage by excision enzyme, and hence the reparability of the damage, may be more efficient in the repressed state than in the active state of a particular gene.

Photoreversal of Suppressors

Suppressor mutations in resistant strains are unusual in their photoreversibility, as well as in their dark repair. Treatments known to inhibit amino-acid-sensitive dark repair (caffeine, acriflavine) can prevent most of the photo-





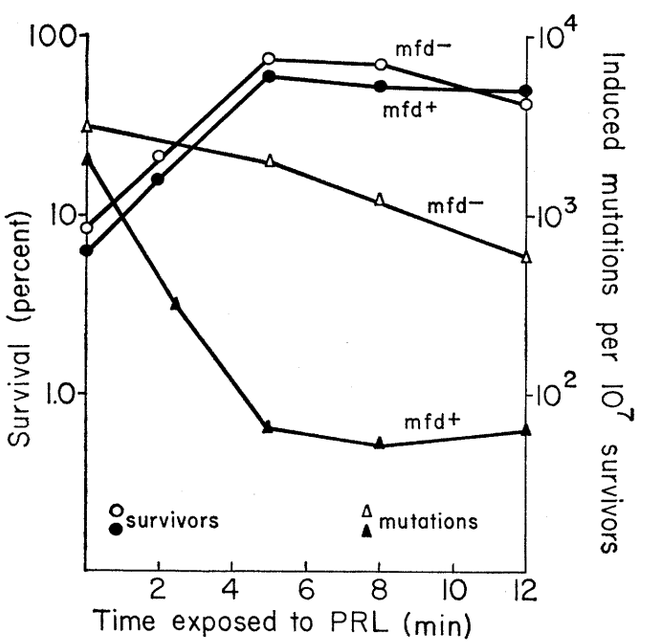


Fig. 7 (top left). "Mutation frequency decline" in strains WU36-10 (*mfd*$^+$) and WU36-10-45 (*mfd*$^-$). Saline suspension of each strain was irradiated with ultraviolet light; diluted 10^{-1} in minimal medium (circles) or nutrient broth containing 25 units of chloramphenicol per milliliter (triangles); incubated times indicated on ordinate, then plated (0.2 ml undiluted) on amino-acid-enriched agar to determine frequency of mutations resulting in independence of tyrosine. Ultraviolet dose: 600 erg/mm^2. Titer before irradiation: *mfd*$^+$, 3.3 × 10^8/ml; *mfd*$^-$, 3.5 × 10^8/ml. Survival: *mfd*$^+$, 11.1 percent; *mfd*$^-$, 12.6 percent. Each point is the average of two experiments.

Fig. 8 (top right). "Mutation frequency decline" in strains WP2 and WP2$_s$. Procedure as described in legend of Fig. 7; suspensions were plated on semi-enriched minimal agar to determine frequency of mutations resulting in independence of tryptophan. Ultraviolet dose: WP2, 850 erg/mm^2; WP2$_s$, 50 erg/mm^2. Survival: WP2, 5.8 percent; WP2$_s$, 7.5 percent. Each point is the average of two experiments.

Fig. 9 (left). Efficiency of photoreversal of potentially lethal damage and induced mutations in strains WU36-10 (*mfd*$^+$) and WU36-10-45 (*mfd*$^-$). Ultraviolet dose: 600 erg/mm^2. PRL= photoreactivating light, exposure immediately after ultraviolet irradiation.

reversal of these mutations, even if the treatments are administered after exposure to the photoreactivating light. Since dimers are split at the instant of exposure to visible light, most of the photoreversal of suppressor mutations (even in strains having photoreactivating enzyme) cannot be direct, enzymatic dimer-splitting. The indirect nature of the photoreversal of suppressor mutations, in resistant strains, is further indicated by the photoreversibility of these mutations in an *mfd*$^-$ strain, selected for loss of the ability to carry out amino-acid-sensitive dark repair. As shown in Fig. 9, the photoreversibility of the potentially lethal effect of ultraviolet light is unchanged, in this strain, an indication that the bacteria are able to split pyrimidine dimers normally. However, the photoreversal of suppressor mutations is much less efficient in this strain than in the *mfd*+ parent strain, from which it differs in lacking the ability to carry out mutation frequency decline. The *mfd*$^-$ mutation duplicates the phenotypic effect of acriflavine (*15*), which also inhibits amino-acid-sensitive dark repair of suppressor mutations and reduces the efficiency of their photoreversal. These results are readily explained by assuming that most of the photoreversal of suppressor mutations, in resistant strains, is really cryptic dark repair. The reactivating light appears to act indirectly, in this case, by creating metabolic conditions that favor mutation frequency decline. The *mfd*$^-$ mutants thus provide additional evidence that suppressor mutations, in resistant strains, arise primarily from photoproducts that are distinguishable, by their reparability, from typical pyrimidine dimers.

Summary

Mutagenesis is compared in an ultraviolet-resistant strain of *E. coli* (WP2) and an ultraviolet-sensitive derivative ($WP2_s$) that lacks detectable ability to excise thymine-containing dimers from its DNA. High frequencies of induced mutations resulting in resistance to streptomycin, independence of tryptophan, and inability to ferment lactose are obtained in the sensitive strain after ultraviolet doses too low to induce significant numbers of such mutations in the resistant strain. It is concluded that potentially excisable photoproducts participate in the induction of all three kinds of mutations; that at least 99 percent of the mutations observed in the sensitive strain are prevented in the resistant strain by repair that includes excision; and that mutations are less likely to occur as "mistakes" in the course of normal repair than as the result of the failure or derangement of normal repair.

At ultraviolet doses having equivalent effects on survival, the sensitive strain exhibits only about half as many mutations to tryptophan-independence, but about ten times as many mutations to streptomycin-resistance, as the resistant strain. This indicates either that repair processes involving excision are not equally efficient in all genes or that some genes contain larger residues of irreparable damage than others, or both.

In the sensitive strain, about 90 percent of the induced mutations to tryptophan-independence and to streptomycin-resistance can be reversed by "direct" enzymatic photoreactivation, that is, by the splitting of pyrimidine dimers. In the same strain, mutations to the inability to ferment lactose are not photoreversible. It is concluded that pyrimidine dimers are involved in the induction of at least 90 percent of the mutations to tryptophan-independence and streptomycin-resistance observed in the sensitive strain, but that prymidine dimers (or at least pyrimidine dimers subject to enzymatic splitting) are not involved in the induction of mutations that result in inability to ferment lactose.

In resistant strains, the ultraviolet-induced mutations arise from a fraction of the photoproducts that is not effectively repaired despite the optimal repair capability of these strains. In the case of streptomycin-resistance, pyrimidine dimers are included in this fraction and participate in the induction of at least 90 percent of the mutations observed in the resistant strain, as shown by their direct photoreversibility. Mutations to prototrophy, in resistant strains, are photoreversible, but mainly by a mechanism not directly involving splitting of pyrimidine dimers. They are repaired in the dark, but only if protein synthesis immediately after irradiation is inhibited (a condition not required for the dark repair of thymine-containing dimers). Although pyrimidine dimers participate in the induction of mutations to prototrophy in the sensitive strain $WP2_s$, most of those observed in resistant strains originate from a residue of photoproducts distinguishable from typical pyrimidine dimers by their pattern of reparability.

Mutations to prototrophy that have unusual repair properties (indirect photoreversal, dark repair blocked by conditions favoring protein synthesis) are invariably suppressor mutations, each capable of correcting many scattered auxotrophic defects, and therefore probably originate in genes coding elements of translations (activating enzymes, transfer RNA's, ribosomes). The unique pattern of reparability exhibited by these mutations may be asssociated with singularities of these loci.

The dark repair of suppressor mutations, in resistant strains, that occurs only if protein synthesis after irradiation is briefly inhibited ("mutation frequency decline") probably involves excision. This inference rests mainly on the reduced rate of dimer excision found in a mutant (*mfd*$^-$) strain selected for the loss of the ability to repair suppressor mutations under conditions normally promoting "mutation frequency decline." It is proposed that suppressor mutations, in resistant strains, arise primarily from photoproducts that can compete successfully with prymidine dimers for excision enzyme only if protein synthesis after ultraviolet irradiation is inhibited.

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38. Research supported by grant AI-01240 from NIAID and conducted with the able assistance of N. A. Sicurella. I thank Dr. L. Gorini for supplying the "conditional streptomycin-dependent" strains, Dr. R. Hill for strain WP2$_s$, and Dr. S. Kondo for the ultraviolet-sensitive *phr*$^+$ and *phr*$^-$ strains (see *22*).

Electrons Accelerated to the 10- to 20-Gev Range

The first full-length operation of the Stanford Two-Mile Linear Electron Accelerator is reported.

W. K. H. Panofsky, R. B. Neal, and the staff of the Stanford Linear Accelerator Center

On 21 May 1966, electrons were accelerated for the first time through the full length of the Stanford Two-Mile Linear Electron Accelerator. Construction of this machine had begun in April 1962. Beam operation with the first two sectors (each sector 333 feet long) had initially taken place on 3 January 1965, and operation with two-thirds of the machine (6700 feet) started on 21 April 1966. The design objective of this machine is to accelerate a maximum electron current of 30 microamperes average to an energy of 20 Gev (10^9 electron volts). Detailed design characteristics of the accelerator may be found elsewhere (*1*).

During the first full-length operation, an energy of about 10 Gev was obtained, with 24 out of the 30 sectors contributing energy but operating at reduced power levels. Subsequently, during the two runs scheduled since that date, the energy has been increased to 16.4 Gev by activating 208 out of the total of 245 klystrons, by improving the phasing adjustments, and by increasing the peak power of the klystrons. Higher-energy operation will be approached cautiously until more experience with the life of the components has been obtained.

Overall Accelerator Performance

Overall accelerator performance to date has been good. Energy measurements have shown that the design goal of 20 Gev should easily be met. The attainable intensity is at present limited to about half the design value of 30 microamperes by the "beam breakup" limit discussed below. Corrective measures are under investigation. Below the beam-breakup threshold, at least 90 percent of the beam measured at a monitor 30 feet from the injector is transmitted through the entire length of the machine. At a pulse repetition rate of 360 pulses per second and a pulse length of 1.5 microseconds, a peak current of 10 milliamperes corresponds to an average current of 5.4 microamperes. With klystrons operating at a conservative output power of 15 megawatts peak, the stability of the machine has been very good. In the absence of any major changes in operating conditions, it has been possible to turn off the beam and reestablish it several hours later without retuning. The automatic phasing system in which the electron beam is used as a phase reference has functioned well. Typical energy spectra with and without beam loading, such as are shown in Fig. 1, have exhibited spectrum widths at half maximum of less than 1 percent. Microwave beam position monitors located at the end of each sector have indicated the transverse beam location with respect to the accelerator axis within ± 0.5 millimeter. Their use has greatly facilitated the functions of steering and focusing the beam along the machine. These functions have been further aided by the use of a 2-mile-long, argon-filled, coaxial line installed along the accelerator. This line works as a continuous ionization chamber and enables the operator to detect beam losses and, from the times of arrival of the ionization signals, to resolve their location within 100 to 200 feet. The capability of the laser alignment system to read out, remotely, the accelerator transverse coordinates to an accuracy of ± 0.010 inch has been demonstrated. Preliminary experiments with interlaced beams such as that illustrated in Fig. 2 have demonstrated the feasibility of transmitting beams of different energies, intensities, and pulse lengths through the accelerator. These beams can then be separated in the beam switchyard for experimental purposes.

Tests on the life of accelerator components, including klystrons, are in progress. In accordance with design,

The Stanford Linear Accelerator Center, Stanford, California, is directed by Dr. Panofsky; Dr. Neal is associate director for the Technical Division.