virus in tube cultures, in contrast to the unsuccessful attempts of Andrewes with suspended fragment cultures(5), perhaps is directly comparable to the experience of Weller with varicella virus. Inoculation of tissue fragments with varicella or herpes zoster materials resulted in inclusion body formation which could not be reproduced on serial passage(13); however, using tube cultures and inocula containing cells, Weller was able to carry the viruses in serial passage(14).

Summary. The guinea pig submaxillary gland virus has been successfully propagated in tissue culture, one strain having been carried through 22 serial passages. The tissue culture passage virus produced characteristic intranuclear inclusion bodies in tissue cultures, demonstrated complement fixing and neutralizing antibody responses in guinea pigs infected with animal passage virus, and produced characteristic disease and histopathology in susceptible guinea pigs. Commercial complement consistently contained high titer CF antibody.

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Differential Photoreactivation of *Escherichia coli* after Exposure to 2650 and 2250 A Ultraviolet.* (23456)

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The authors have reported(1) that the fraction of the mutagenic and lethal effects of ultraviolet (UV) radiation in *Escherichia coli* cells that is photoreactivable is the same after exposure to 6 wave lengths ranging from 2378 to 2967° A. The fraction of the effect that was photoreactivable was significantly greater for mutation than for inactivation. Extension of these studies to 2250 and 2180 A has revealed some interesting differences in the response to photoreactivating (PR) light and in the response of different strains of *E. coli*.

Inactivation and mutation to streptomycin independence in *E. coli* SD-4 was studied initially. *E. coli* SD-4 is particularly well suited for tests of photoreactivation of mutations since a linear mutation-dose relation is obtained(1,2). The methods of preparation of cells for irradiation and of exposure to UV and PR light have been described(1). Muta-

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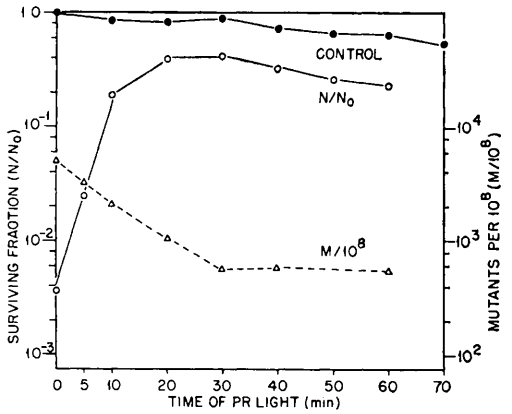

FIG. 1. Survival of *Escherichia coli* strain SD-4 after irradiation with 2650 A ultraviolet.

tion and growth assay methods were similar to those of Anderson(2). For photoreactivation, the samples were placed in small Petri dishes and exposed at 37°C at a distance of 20 cm to the radiation from an AH-5 250-watt mercury arc lamp. The glass envelope was not removed from the lamp and the radiation was filtered through a 5-cm layer of 10% $CuSO_4$ to absorb infrared radiation.

Results. Typical photoreactivation curves of survival ratio and mutants per 10^8 surviving cells are shown in Fig. 1 for SD-4 cells exposed to 2652 A ultraviolet. Similar curves are obtained for 5 other wave lengths between 2378 and 2967 A. The photoreactivation curve for mutation is similar to that for inactivation with an approximately exponential decrease in mutants per 10^8 reaching a minimum (maximum photoreactivation) at about 25 to 30 min exposure, after which the number remains essentially constant.

After exposure of SD-4 to 2250 UV, a quite different response to PR light is observed (Fig. 2). The survival ratio increases initially after exposure to PR light until a maximum is reached at 10 min exposure. Then there is an approximately exponential decrease in survival ratio during continued exposure of the cells to PR light. This decrease in survival ratio is significantly steeper in slope than that experienced by the control cells during exposure to PR light and has been observed repeatedly after exposure to 2250 and 2180 A UV. It appears that these shorter wave lengths in some manner sensitize the SD-4 cells to PR light that, under our conditions, includes wave lengths in the range of about 3100 A up through the visible spectrum. No effort was made to determine which wave lengths in this region were effective in producing this post-UV inactivation. The photoreactivation curve of mutants per 10^8 surviving cells again is quite similar to that for inactivation, with a minimum at 10 min exposure corresponding to the maximum for photoreactivation of the survival ratio. The mutants/10^8 survivors may increase with continued exposure to PR light, but this is not certain.

It appears that the magnitude of photoreactivation of survival ratio after 2250 A UV is actually less than after exposure to 2652 A ultraviolet. The 2250 A photoreactivation survival curve is however a resultant of 2 opposing processes, photoreactivation of UV-inactivated cells and inactivation of survivors of the UV exposure by the PR light. No attempt has yet been made to quantitate these opposing effects of PR light but it is obvious that the actual maximum of photoreactivation is greater than that indicated by the peak of the curve in Fig. 2, and may approach in magnitude that exhibited by cells after exposure to longer UV wave lengths.

The response to PR light of a number of other strains of *E. coli* was studied to deter-

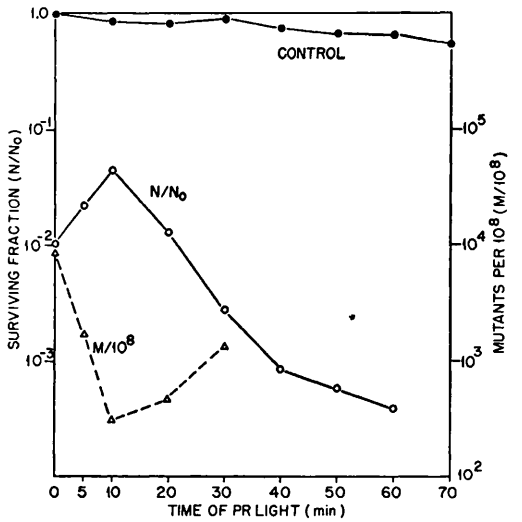

FIG. 2. Survival of *Escherichia coli* strain SD-4 after 2250 A ultraviolet.

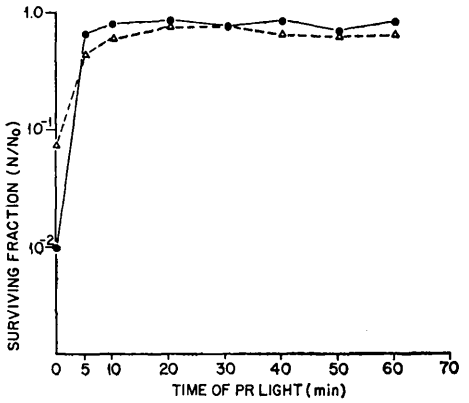

FIG. 3. Survival of *Escherichia coli* strain 42-24 after 2650 and 2250 A ultraviolet.

mine if the behavior exhibited by *E. coli* SD-4 was unique. SD-4 is a streptomycin-dependent mutant of *E. coli* B/r(3), the radiation-resistant mutant of *E. coli* strain B isolated by Witkin(4). *E. coli* strain 82/r is a purine-requiring auxotrophic mutant of *E. coli* B/r that was also employed by Anderson(2) in his studies of X-ray-induced mutations. Survival of B/r and 82/r behaves quite similarly to survival of SD-4 exposed to PR light after 2250 and 2652 A UV. Induced back-mutations in 82/r parallel the survival ratio in response to PR light, but it is known(1) that the apparent rate of mutation in 82/r is subject to other influences than UV dose.

A quite different response to PR light was noted after exposure of *E. coli* strain 42-24 to 2250 A ultraviolet (Fig. 3). Strain 42-24 is a serine-glycine-requiring auxotroph obtained from Dr. R. Girolami and used by him in studies of X-ray-induced back-mutations in which a linear mutation-X-ray dose response curve was observed (personal communication). With strain 42-24, almost identical photoreactivation of inactivation is observed after exposure to 2652 and 2250 A UV. There is no sensitization to PR light after exposure to 2250 A UV. Strain 42-24 also differs from SD-4 in that the maximum of photoreactivation is reached more quickly and the slight decrease in survival ratio observed with SD-4 control and 2650 A-irradiated cells seems to be absent during the 60-min exposure of 42-24 cells to PR light. The mutagenic response of strain 42-24 to UV is so small that statistically accurate back-mutation data have not yet been obtained.

Unfortunately, the origin of strain 42-24, which was added to the culture collection of the Biology Division of the Oak Ridge National Laboratory by the late Dr. E. H. Anderson, is unknown. However, judging from its growth characteristics, radiation response, and motility, we are quite certain that it is not a derivative of *E. coli* B or B/r.

Conclusion. Our data establish that photoreactivation of both inactivation and mutation occurs after exposure of *Escherichia coli* SD-4 to 2250 and 2180 A UV. The magnitude of the photoreactivation after exposure to short-wave-length ultraviolet in *E. coli* B/r and its derivatives is confounded with a short-wave-length UV sensitization to inactivation by the PR light itself. The quite different response to photoreactivating light exhibited by *E. coli* strain 42-24, presumably not derived from *E. coli* B/r, suggests that different strains may differ in response to UV and PR light. It would seem prudent, therefore, to study a number of unrelated strains before generalizations are made concerning mechanisms of radiation action in bacteria.

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