REPAIR IN PHAGE AND BACTERIA INACTIVATED BY LIGHT FROM FLUORESCENT AND PHOTO LAMPS

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Summary: Phage P22 of Salmonella typhimurium were inactivated by light from a variety of household fluorescent and photo lamps. The damage (presumably to DNA) was repaired when phage were grown in hcr$^+$ cells (host cell reactivation), but not in hcr$^-$ cells. UV germicidal wavelengths (ca. 2537 Å) produced the same killing effect on phage as that from wavelengths in the visible (above 4000Å) ranges. However, unlike phage, when various bacterial mutants were irradiated, the effects from visible and germicidal lamps were quite different from each other. We conclude that visible and near-visible wavelengths can produce two kinds of damage: (1) directly on DNA, presumably pyrimidine dimerization, and (2) an alteration other than dimerization.

Bacterial mutants of Salmonella typhimurium have been found that are highly sensitive to light emitted from a variety of household and photo lamps (Eisenstark, 1969). Interestingly, these mutants are unable to undergo genetic recombination (rec) and are also sensitive to UV, X-ray, and a variety of chemical mutagens (Eisenstark, et al, 1969).

In addition to these highly sensitive mutants, other S. typhimurium mutants have been found that are also light sensitive, but to a much lesser degree (Eisenstark, 1969; Webb and Lorenz, 1969) (Fig. 1). These bacterial mutants are unable to repair UV damage to phage (hcr) and are themselves sensitive to UV, but not to X-ray or chemical mutagens.

These observations bring specific questions into focus: (1) Is there identical damage from visible light as from UV? (2) Is pyrimidine dimerization in DNA the only important damage to the cell? (3) If not, what is the second altered molecule (or the second alteration in the DNA) that is ultimately repaired in normal cells?

Although DNA is highly reactive to UV and X-ray, it has not been

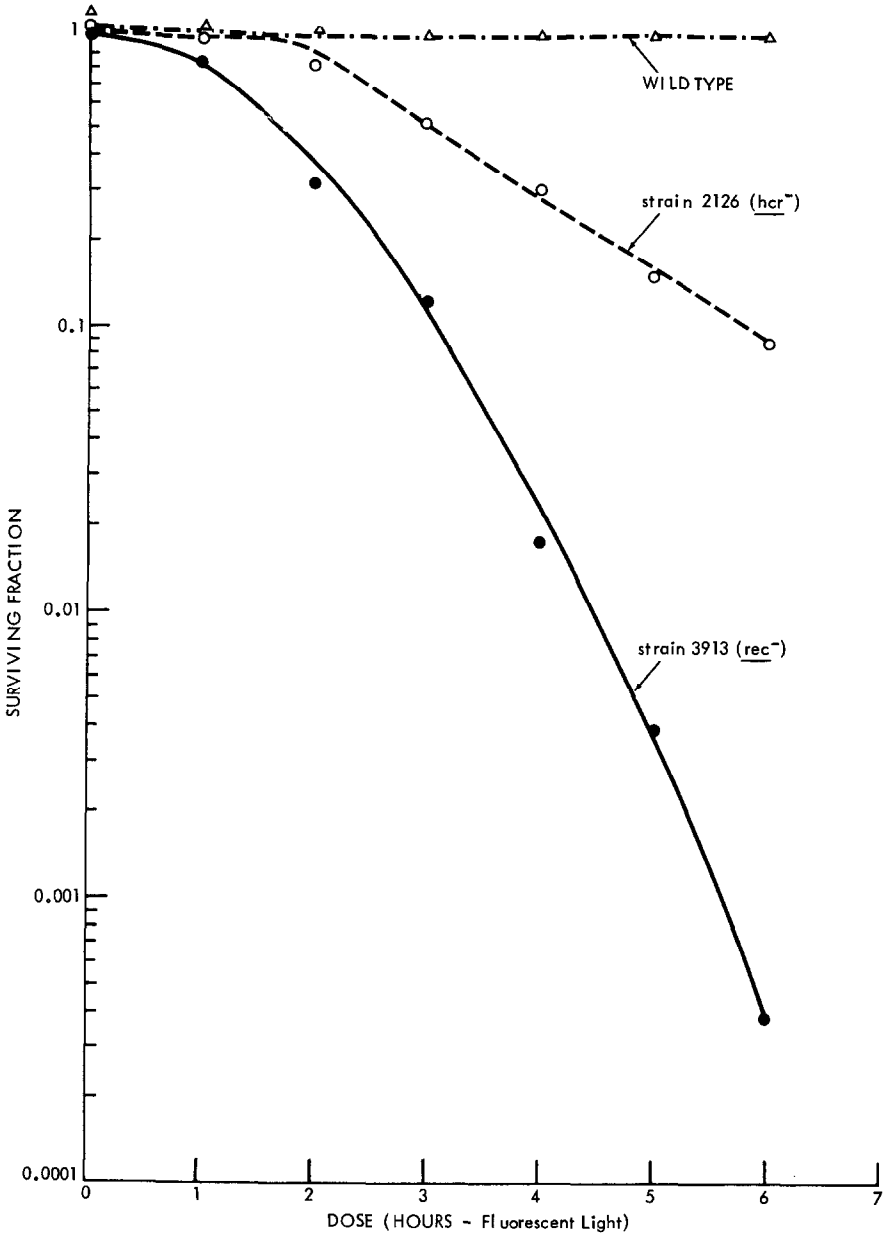

Fig. 1. Killing of S. typhimurium by 15-watt fluorescent lamp (GE cool white F15T8CW). Close circles (●——————●) represent mutant strain 3913, rec-7 trpABEI30 thy-779; open circles (O————————O), strain 2126, hcr-24 thy-177; triangles (▲-·-·-·-·-·-·-▲), wildtype strain. Overnight cultures were diluted 10^{-2} in fresh nutrient broth, shaken at 37 C for two hours to bring cells into log phase, and then diluted again 10^{-2} in nutrient broth. Samples (10 ml) were placed in small plastic petri dishes, stirred magnetically, and exposed to lamp 3 inches above surface (70 foot-candles, as measured with GE lightmeter). Samples were removed periodically and plated on nutrient agar for colony counts.

While Fig. 1 presents results with 15-watt fluorescent bulb, a series

of other lamps were used in parallel experiments (Sylvania F 15T8BL blacklight bulb with spectra that peak at 3650 Å; 500-watt photoflood lamp; strobe light, Honeywell Strobonar 400; and 500-watt iodine-quartz lamp in a 35 mm slide projector, Montgomery Ward 777AQ); inactivation curves led to identical conclusions as those delineated by Fig. 1. Also, each of these lamps were used with filters (Jagger, 1967) in which either the near UV (under 4000 Å) or the visible range (over 4000 Å) was eliminated. The results paralleled those in Fig. 1, which indicates that visible and near visible wavelengths yield similar results.

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seriously considered as a vulnerable macromolecule for wavelengths of 3650 Å and above (Jagger, 1967), despite early studies with long UV which showed lethality to phage (Wahl and Latarjet, 1948). Recent experiments, however, emphasize that DNA is affected by visible and near-visible light, and it now needs to be resolved whether at least some of the observed biological effects are the result of damage other than pyrimidine dimerization. In the observed lethal and mutagenic action of light on bacterial cells (Eisenstark, 1969; Webb and Malina, 1967; and Kubitschek, 1967) the altered molecule is not identified. However, since visible light can modify the transforming capacity of DNA and inactivate phage (Cabrera-Juarez, 1964; Wahl and Latarjet, 1948), it is obvious that there is a direct DNA alteration. Although less convincing than the transformation experiment, further suggestion of a direct light effect on DNA is gained from the knowledge that the light is most effective during the period of chromosome replication in the bacterial growth cycle (Eisenstark, 1969).

This report presents evidence that visible and near-visible wavelengths have direct effect on phage DNA. We exposed phage P22 of Salmonella typhimurium to a variety of household and photo lamps. As may be seen in Fig. 2, phage lost ability to produce plaques when plated on mutant hcr cells (host cell reactivation); however, damage by light was repaired when phage infected hcr^+ cells.

It is believed that hcr mutants are unable to remove pyrimidine dimers formed by UV exposure (Howard-Flanders, 1968). From the results of our experiments, presumably, visible and near-visible light alter the DNA

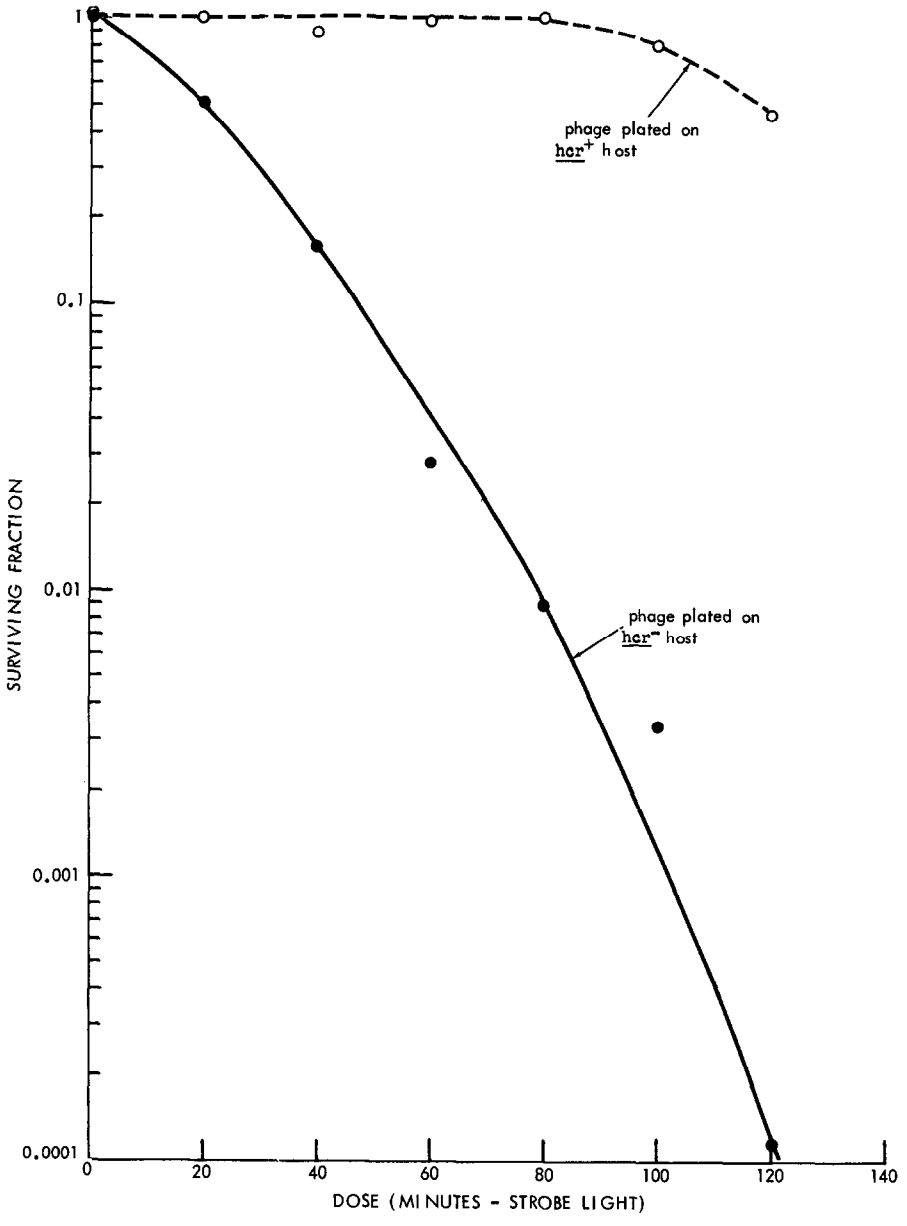

Fig. 2. Inactivation of phage P22 by strobe light (Honeywell Strobonar 400) and host cell reactivation by S. typhimurium. Closed circles (●——————●), represent surviving fractions when phage were plated on strain 2126, hcr-24 thy-177. Open circles (O----------O), surviving fractions when phage were plated on hcr$^+$ host. Phage samples in physiological saline (ca. 10^6/ml) were placed in small plastic petri dishes, stirred magnetically and exposed to the strobe light at a distance of three inches above surface. Flashes were spaced 15 seconds apart. Samples were removed periodically and plated on nutrient agar. All of the other lamps listed in Fig. 1 yielded similar results when tested in parallel experiments.

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of phage in a manner similar to UV and the defect (pyrimidine dimers?) can be removed by the wildtype cell but not by the hcr mutant.

Although the reactivation of damaged phage is the same regardless of whether the irradiation source is UV or visible light, the two light sources act very differently when bacteria are irradiated. UV inactivates both hcr and rec cells to relatively the same extent, but only the rec strains have high sensitivity to visible light (Figs. 1 and 2). Two alternative explanations may be considered. (1) Phage damage by light may be limited to DNA pyrimidine dimerization which rec cells can repair; bacterial damage may involve a second alteration which rec cells cannot repair. (2) Both phage and bacterial DNA receive two kinds of damage; rec cells can remove dimers in both but lack a repair gene for the second damage. The phage, however, has its own repair gene for this second damage. This does not clarify whether the second damage to rec cells by visible and near-visible light is the result of a DNA alteration. We are now searching for an altered (non-DNA) molecule in rec that could explain sensitivity to light.

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