0022-202X/81/7701-0091$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 77:91–95, 1981

Vol. 77, No. 1
Printed in U.S.A.

Photoreactivation in Bacteria and in Skin

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In many procaryotic and eucaryotic cells, photoreactivating enzyme mediates light-dependent repair of UV-induced damage: the enzyme binds to a pyrimidine dimer in DNA, and, on absorption of a photon (300–600 nm), specifically monomerizes the dimer, thus repairing the DNA.

Photoreactivating enzyme has been found in human tissues and human cells in culture; human cells in culture can photoreactivate cellular dimers, and can mediate photoreactivation of Herpes (human fibroblasts) and Epstein-Barr virus (human leukocytes). Measurements of pyrimidine dimer formation and repair in human skin indicate that detectable numbers of dimers are formed at 1 minimal erythemal dose, that the dimers are rapidly removed in skin kept in the absence of light, and they are more rapidly removed when the skin is exposed to visible light.

Photoreactivation was first known as a biological recovery phenomenon [1,2], and only gradually are its cellular mechanisms and molecular architecture being understood. It is manifested as a biological effect by a reduction of UV-induced biological damage by light (300–600 nm) administered *after* UV (220–320 nm) exposure. The first knowledge of the cellular mechanism of photoreactivation came with the pioneering work of Rupert, who showed that UV-irradiated DNA was the substrate for a photoreactivating enzyme (PRE) which formed an enzyme-substrate complex (with UV-irradiated DNA as substrate) and obeyed Michaelis-Menten kinetics [3,4]. He showed that the enzyme-substrate complex was stable until exposed to photoreactivating light; upon absorption of the light, the DNA was repaired and the enzyme was released (Fig 1). The next major advances were the demonstrations by Wulff and Rupert [5], and by Setlow, Boling, and Bollum [6] that the pyrimidine dimer in DNA was a substrate for the enzyme, and by Setlow and Setlow [7] that the dimer was the *only* substrate for the PRE.

Three essential ingredients distinguish enzymatic photoreactivation from other repair or recovery events. (1) Amelioration of UV-induced damage is mediated by light in the wavelength range 300–600 nm. This allows exclusion of direct photoreversal of dimers by 200–300 nm light which, in very UV-resistant systems, can result in increased biological survival. (2) The requirement of the temporal sequence of UV *then* photoreactivating light excludes photoprotection, in which longer wavelength light administered *before* UV can increase survival [8]. (3) The presence of biological photoreactivation must be correlated with the presence of a photoreactivating enzyme. In addition to direct photoreversal and photoprotection, several other recovery phenomena can be distinguished from photoreactivation through use of these 3 criteria. For example, in photosynthetic organisms, exposure to "photoreactivating" light can alter cellular metabolic processes relative to those in cells kept in the dark. Even in nonphotosynthetic organisms, heat from photoreactivating lights might increase the rate of repair by other processes. Thus, it is essential to examine putative "photoreactivation" processes to be certain that they are not in truth mediated by some other process; it is also important to examine carefully photoreactivation conditions in cases where no photoreactivation was found. Inadequate exposure times, light intensities, incorrect wavelength distribution—all these can prevent the observation of photoreactivation.

PHOTOREACTIVATION IN BACTERIAL CELLS

Photorecovery in bacteria can be shown to meet all these criteria. The best-studied organism is *Escherichia coli*. (Note that not all bacteria are photoreactivable; the naturally transformable bacteria neither contain photoreactivating enzyme nor carry out cellular photoreactivation.) In *E. coli*, exposure of UV (e.g., 254 nm) irradiated cells to radiation in the wavelength range 300–500 nm (see Fig 2) leads to increased biological survival and decreased mutation [9]. Second, this photorecovery event depends on the administration of photoreactivating light after the UV exposure. Third, a photoreactivating enzyme can be isolated from *E. coli* cells, and its presence and absence are correlated with the presence and absence of photoreactivation* [10].

Further, the light-dependence of the photolysis reaction allows the determination of its "quantum signature," its action spectrum or wavelength dependence of photoreactivation [11]. Although all action spectra for photoreactivation thus far determined have a short wavelength cut-off of about 300 nm (probably because shorter wavelengths also form dimers), their maxima and long wavelength edges vary widely. This characteristic "signature" allows the determination of whether a biological photorecovery event is photoreactivation or some other recovery process. Figure 2 shows that the action spectrum for cellular photoreactivation in *E. coli* is an excellent reflection of that for *in vitro* action of the isolated *E. coli* enzyme [12].

The specificity of the photoreactivating enzyme for pyrimidine dimers in DNA [12] allows an additional handle on the examination of a photorecovery event. If biological recovery results from photoreactivation, the monomerization of pyrimidine dimers should accompany the biological recovery event. In *E. coli* exposed to rather large UV doses, it is easy to show that dimers are monomerized during photoreactivating light treatment; the difficulty of measuring dimers at the low dimer concentrations present after doses which sensitive cells can survive has been overcome by the development of new gel techniques adequate for the detection of, for example, 1–2 breaks per 50×10^6 d [13,14].

PHOTOREACTIVATION IN CULTURED MAMMALIAN CELLS AND MAMMALIAN

By 1970 photoreactivation in lower and in many higher eucaryotes had been well established by biological, biochemical, and biophysical tests. However, the only mammalian cells in

This work was supported by grants (CA23096 and CA26492) and a Research Career Development Award (CA00466) from the National Cancer Institute of the NIH to BMS, and the Department of Energy.

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Abbreviations:
PR: photoreactivation
PRE: photoreactivating enzyme
XP: xeroderma pigmentosum

* Cells which possess efficient "dark repair" mechanisms may show only small photoreactivation effects, probably due to competition of the dark repair enzymes and (PRE) photoreactivating enzyme molecules for the same lesions in DNA.

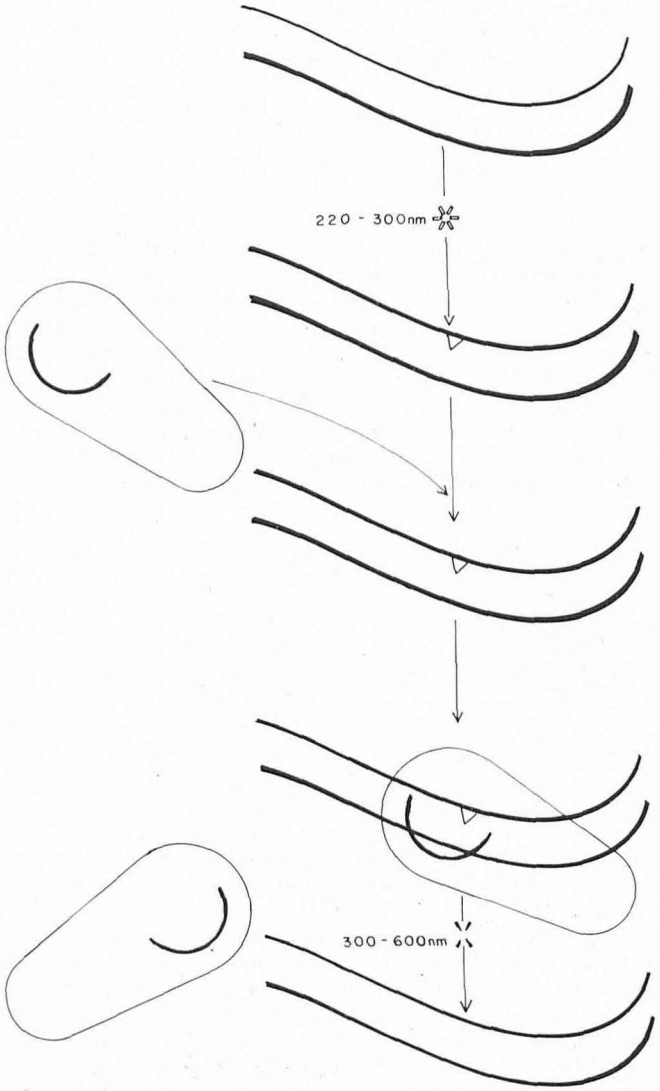

FIG 1. Action of the photoreactivating enzyme. DNA absorbs an ultraviolet photon, and a pyrimidine dimer is formed. The photoreactivating enzyme binds to the dimer-site, and on absorption of a photon (300–600 nm) monomerizes the dimer and is released from the DNA.

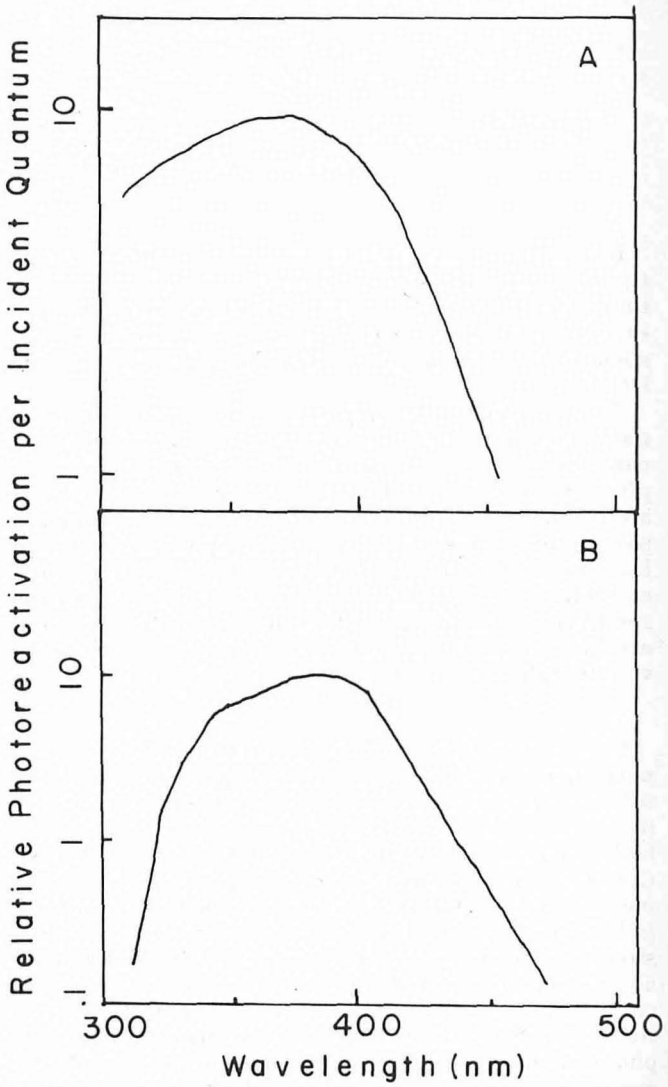

FIG 2. Action spectra for photoreactivation in *E. coli* cells (*upper curve*) (reference 37) and by the isolated *E. coli* enzyme (*lower curve*) (reference 11).

which it had been detected were those of the marsupial *Potorous tridactylus* [15]. After the reports by Sutherland [16] and by Harm [17] of a photoreactivating enzyme activity in cells of placental mammals, it was of major importance to determine whether this activity and its cellular manifestations met the criteria for photoreactivation *in vitro* and *in vivo*.

The first criterion is that the wavelengths effective in the photorecovery be greater than 300 nm (to exclude direct photoreversal). Photoreactivating enzyme activity *in vitro*, photoreactivation of cellular dimers, and biological photoreactivation all utilize light greater than 300 nm (see also below) [18–20]. Second, these activities and photorecovery effects must meet the temporal requirement of effectiveness of the photoreactivating light only when it follows UV exposure. For both the enzyme activity and cellular photorecovery, photoreactivating light given before UV is ineffective while that given after the UV readily drives the photoreactivation reaction. The third criterion demands the association of photoreactivation with the presence of photoreactivating enzyme. As mentioned above, the presence of other efficient repair systems can mask or prevent the action of a photoreactivating enzyme. Thus human excision-deficient xeroderma pigmentosum (XP) cells provide a good test system for examining photoreactivation. Wagner, Rice, and Sutherland measured photoreactivation of UV-inactivated Herpes virus in 2 XP cell strains of similar excision capacity but differing in PRE activity [20]. They were able to detect photoreactivation of Herpes plaque-forming ability in XP12BE, which contained PRE at a specific activity of about 220 pmol/mg/hr, but not in XP1LO, with a specific activity of only about 3 pmol/mg/hr. (They could not detect PR of Herpes by normal human fibroblasts, even though they contained high levels of PRE activity, presumably due to competition from the host's excision system. Similar results were obtained by Henderson, who found photoreactivation of Epstein-Barr virus in XP cells but not in excision proficient XP heterozygotes [21].)

Just as in the bacterial PR (photoreactivation) systems, the wavelength dependence of the mammalian PRE provides a powerful tool for identifying cellular photorecovery events as photoenzymatic repair. Figure 3 (*lower curve*) shows the action spectrum for photoreactivating enzyme action *in vitro* and (*upper curves*) that for cellular dimer monomerization [19]. These action spectra are similar in maximum and range, indicating that the photocatalyzed disappearance of dimers from the cells indeed results from action of the enzyme.

Since PR action spectra are broad and structureless, their most distinguishing features are their maximum and long-wavelength extent. Although the maximum (400 nm) of the human PR action spectrum is unremarkable (other action spectra have

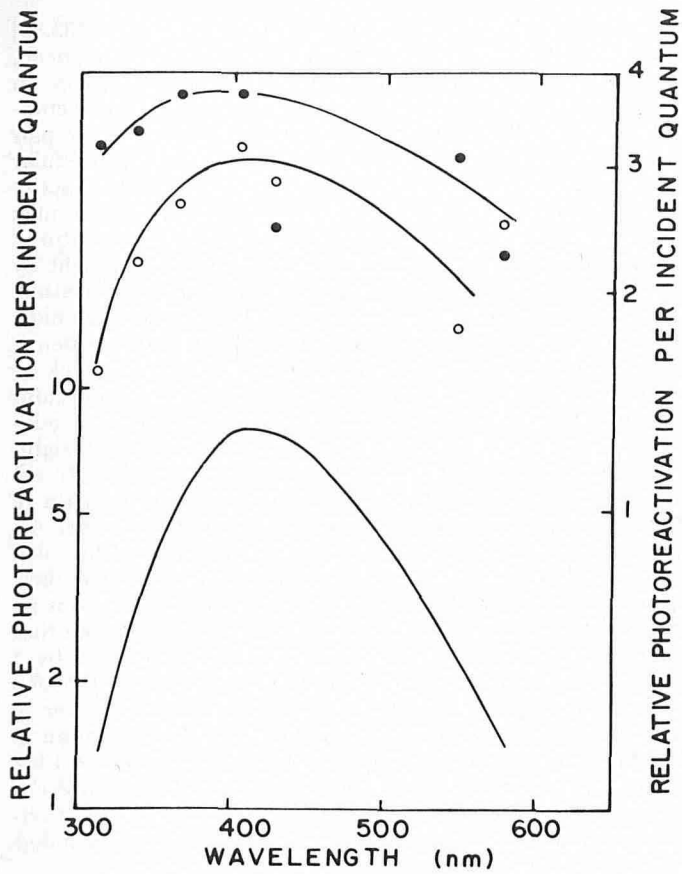

FIG 3. Action spectra for photoreactivation by isolated human photoreactivating enzyme (*lower curve*) and for cellular dimer photoreactivation by human cells in culture. Reprinted with permission from Sutherland BM, Oliver R, Fuselier CO, Sutherland JC: Biochemistry 15:402–405, 1976. Copyright 1976, American Chemical Society.

maxima ranging from 360 to 436 nm), the extension of the spectrum to wavelengths greater than 500 nm had not been seen in other action spectra. Speculation on selective pressures for utilization of the wavelengths between 500 and 600 nm is interesting, but of more immediate practical importance is that the gold lights used as "safelights" for most photoreactivating enzyme studies are not safelights at all for human cells and in fact can drive the PR reaction [18]. Evidently the ability to use light of longer wavelengths is not confined to the PRE from placental mammals; recently Chiang and Rupert have shown that enzyme from the marsupial *Potorous* can also utilize light of wavelength greater than 500 nm [22].

PHOTOREACTIVATION IN SKIN

The major photorecovery phenomenon which has been studied in human skin is reduction of shorter-UV induced erythema by longer wavelength light. Although Van der Leun and Stoop reported photorecovery of 250 nm—or 300 nm—irradiated skin to filtered sunlight, [23] neither Willis, Kligman, and Epstein [24] nor Ying, Parrish, and Pathak [25] observed such recovery. More recently Van Weelden has observed photorecovery from erythema, in fact a larger effect than that observed by Van der Leun [26]. The reasons for the contradictory results are not yet clear. It is also not clear that the erythemal response results from pyrimidine dimer formation or that the light-induced recovery observed by Van der Leun and Stoop and by Van Weelden results from photoenzymatic repair. Although action spectroscopy may offer an answer to these questions, direct comparisons of action spectra for cells in the lower levels of the epidermis or in the dermis with those of possible absorbers are complicated by the "filter effect" of upper skin layers. (See Cooke and Johnson [27] for an attempt to deconvolve the absorption of the filter of upper skin-layers from absorption leading to pyrimidine dimer formation in skin.)

Pyrimidine dimer measurements in skin have been limited by the requirement for high specific radioactivity in DNA for dimer determination by conventional methods. Thus dimer measurements were confined mainly to rodent skin [27–31]. Several investigators have also examined removal of dimers in UV-irradiated rodent skin. Bowden et al [29] as well as Cooke and Johnson [27] found removal of dimers from skin of UV-irradiated mice. However, Ley, Sedita, and Grube [31] detected neither excision nor photoreactivation in the skin of adult hairless mice, although embryonic mouse cells in culture undergo slow excision [32] and contain low levels (~10% that of human embryonic fibroblasts grown under the same conditions) of photoreactivating enzyme [19]. Ananthaswamy and Fisher have detected apparent photoreactivation of pyrimidine dimers in the skin of neonatal mice [33]. They have suggested that the apparent conflict between their results, those obtained from culture and those of Ley, Sedita, and Grube may reflect the use of adult mice by Ley and his collaborators, and embryonic cells or neonatal mice in the other studies.

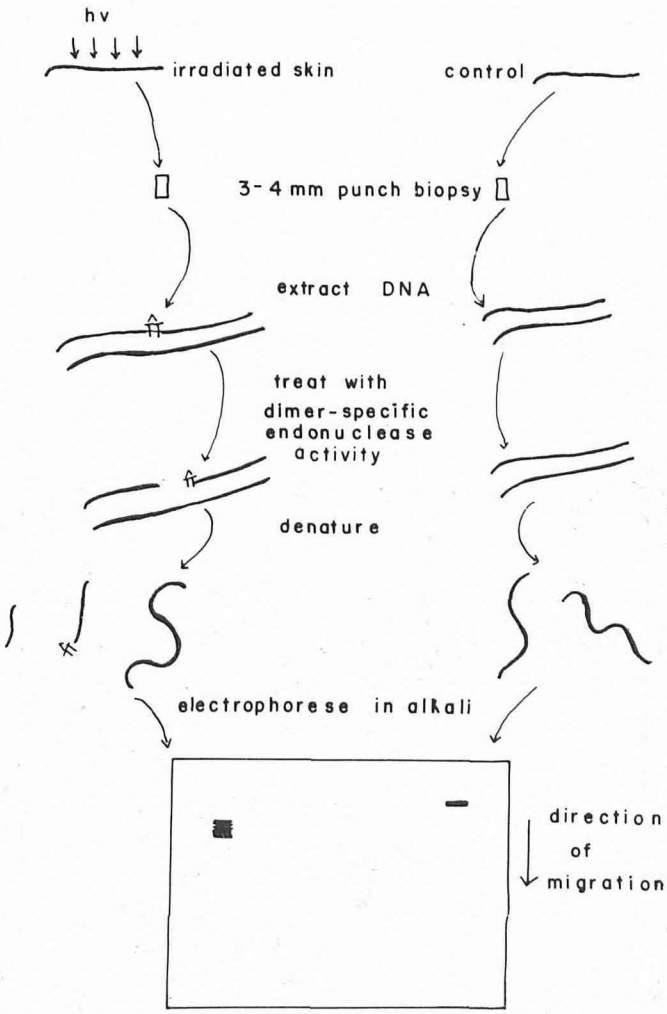

FIG 4. Pyrimidine dimer measurement by alkaline agarose gel electrophoresis. Cells are untreated (*left column*) or UV-irradiated (*right column*). The cellular DNA is extracted, and treated with an endonuclease which makes a single-strand nick adjacent to each dimer. The DNA is denatured in alkali and the molecules separated according to molecular weight by alkaline agarose gel electrophoresis. The DNA is neutralized, stained with ethidium bromide, photographed and the negative scanned in a densitometer.

Epstein et al examined UV-induced unscheduled DNA synthesis, a measure of excision repair, in the skin of normal individuals and xeroderma pigmentosum patients [34]. They found that repair synthesis was detectable within 1 hr in normal skin, but did not occur in the skin of the XP patient.

Although these experiments were carried out with a radioactive label, it would be difficult today to obtain adequate specific radioactivities in human skin DNA to allow quantitative dimer determinations, especially at low UV doses of biological significance. Sutherland, Harber, and Kochevar [35,36] thus adapted to human skin the method of Achey, Woodhead, and Setlow [14] for measurement of pyrimidine dimers in nonradioactive DNA. Figure 4 shows a schematic representation of this method: cells are treated with UV, allowed to repair or kept untreated according to experimental protocol. Cellular DNA is extracted and treated with a "UV-endonuclease" activity from *Micrococcus luteus*, which makes a single-strand nick adjacent to each pyrimidine dimer [37]. The DNA is denatured and separated according to single-strand molecular weight by electrophoresis in an alkaline agarose gel. After neutralization, the DNA is stained with the fluorescent dye ethidium bromide, photographed, and the resulting negative scanned in a densitometer. The presence of pyrimidine dimers leads to a nick by the enzyme and thus a reduction in the single-strand molecular weight of the DNA; dimer removal results in fewer "UV endonuclease" sites and a resulting increase in molecular weight. Sutherland, Harber, and Kochevar used this technique to examine dimer content of DNA from untanned gluteal skin of healthy volunteers which was untreated (Figure 5, *panel A*), exposed to 1 MED of FS-20 sunlamp radiation and biopsied immediately (*panel B*), at 20 min postirradiation in the dark (*panel C*) or after 20 min exposure to visible light from an incandescent bulb (*panel D*). These experiments indicate that approximately 10 dimers per 10^8 d DNA were formed by 1 MED of sunlamp exposure, that 40% of these were removed after about 20 min in the dark, and 60% were removed after 20 min exposure to photoreactivating light. Photoreactivation in human skin has also been reported by D'Ambrosio et al [38]. Although these results strongly suggest that the photoreactivating enzyme present in human tissues is active in skin, alternate mechanisms of photorecovery have not yet been excluded.

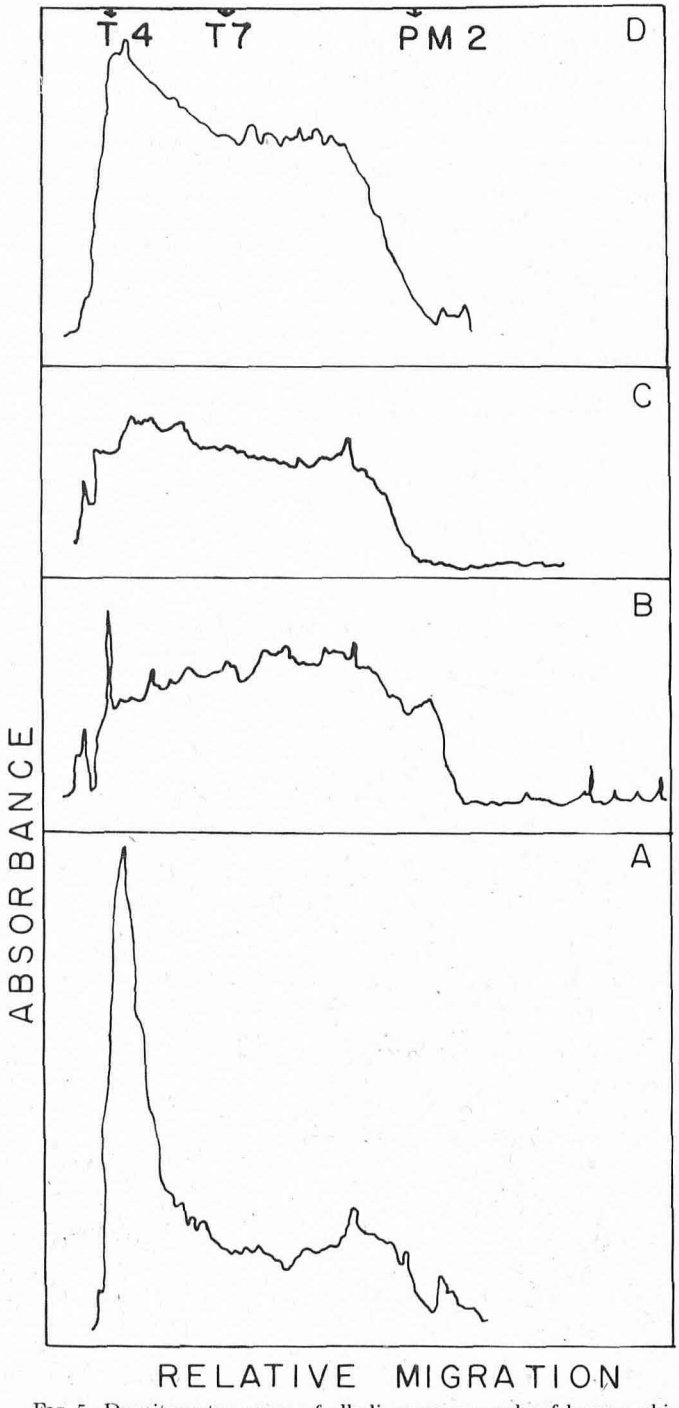

FIG 5. Densitometer scans of alkaline agarose gels of human skin DNA. Biopsies were obtained from untreated skin (*panel A*), skin exposed to 1 MED of FS-20 sunlamp radiation and biopsied immediately (*panel B*), at 20 min postirradiation in the dark (*panel C*) or after 20 min exposure to visible light (*panel D*). Reprinted with permission from Sutherland BM, Kochevar I, Harber L: Cancer Res in press, 1980.

PROSPECTS FOR FUTURE RESEARCH

It is clearly essential to ascertain whether the apparent photoreactivation of dimers results from enzymatic photoreactivation. Two straightforward approaches to this problem are (1) the examination of photorecovery in skin of individuals with lowered PRE levels and (2) the measurement of an action spectrum for the photomonomerization of dimers and comparison with that for action of the human enzyme. As mentioned above, the action spectral comparisons may be complicated by overlying cellular layers. An equally important problem is that of the relation of dimer biochemistry and the biology of UV damage: are dimers involved in the production of erythema? In ultraviolet oncogenesis? Is the apparent photorecovery from erythema a reflection of photoenzymatic monomerization of dimers? Here again, the combination of action spectroscopy and judicious choices of biological systems may provide direct answers to difficult questions of primary importance.

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