

BNL 28385
BIO 3642

Conf Art

CONF-80/0102 --1

PHOTOREACTIVATION IN BACTERIA AND IN SKIN

Betsy M. Sutherland, Ph.D.

Department of Biology

Brookhaven National Laboratory

Upton, NY 11973 USA

Send proofs and reprint requests to author and address above.

Running head: Photoreactivation

MASTER

DISCLAIMER

This book was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

eb

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

ABSTRACT:

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 erythema 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. Whether this apparent photorecovery is true, enzymatic photoreactivation is yet to be determined.

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 (Figure 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 that the dimer was the only substrate for the PRE.

Three essential ingredients distinguish enzymatic photoreactivation from other repair or recovery events. First, 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. Second, the requirement of the temporal sequence of UV then photoreactivating light excludes photoprotection, in which longer wavelength light administered before UV can increase survival [7]. Third, 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 three 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 non-photosynthetic 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 Figure 2) leads to increased biological survival and decreased mutation [8]. 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 photoreactivating enzyme*[9].

Further, the light-dependence of the photolysis reaction allows the determination of its "quantum signature", its action spectrum or wavelength dependence of photoreactivation [10]. 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

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

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 [11].

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.

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 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, 19, 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 two 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 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 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 erythematous 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, 28, 29, 30, 31]. Several investigators have also examined removal of dimers in UV-irradiated rodent skin. Bowden, Trosko, Shapas and Boutwell, [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.

Epstein, Fukuyama, Reed and Epstein 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 one hour 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] 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 [36]. 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. 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.

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.

ACKNOWLEDGMENTS:

Research 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.

REFERENCES

- [1] Kelner A: Effect of visible light on the recovery of Streptomyces griseus conidia from ultraviolet irradiation injury. Proc. Natl. Acad. Sci. US 35(2):73-79, 1949.
- [2] Dulbecco R: Experiments on photoreactivation of bacteriophages inactivated with ultraviolet radiation. J. Bacteriol 59:329-347, 1950
- [3] Rupert CS: Photoenzymatic repair of ultraviolet damage in DNA. I. Kinetics of the reaction. J. Gen. Physiol. 45:703-724, 1962
- [4] Rupert CS: Photoenzymatic repair of ultraviolet damage in DNA. II. Formation of an enzyme-substrate complex. J. Gen. Physiol. 45:725-741, 1962
- [5] Wulff DL, Rupert CS: Disappearance of thymine photodimer in ultraviolet irradiated DNA upon treatment with a photoreactivating enzyme from Baker's yeast. Biochem. Biophys. Res. Commun. 7:237-240, 1962
- [6] Setlow JK, Boling ME, Bollum FJ: The chemical nature of photoreactivable lesions in DNA. Proc. Natl. Acad. Sci. USA 53:1430-1436, 1965
- [7] Jagger J, Stafford RS: Biological and physical ranges of photoprotection in microorganisms. Photochem. Photobiol. 1:245-257, 1962

- [8] Jagger J, Takebe H, Snow JM: Photoreactivation of killing in Streptomyces: Action spectra and kinetic studies. Photochem. Photobiol. 12:185-196, 1970
- [9] Sutherland BM, Court D, Chamberlin MJ: Studies of the DNA photoreactivating enzyme from Escherichia coli.
I. Transduction of the phr gene by bacteriophage lambda Virology 48:87-93, 1972
- [10] Jagger J: Introduction to research in ultraviolet photobiology. Prentice-Hall, Englewood Cliffs, NJ, 1967
- [11] Sutherland BM, Chamberlin MJ, Sutherland JC: Deoxyribonucleic acid photoreactivating enzyme from Escherichia coli. J. Biol. Chem. 248:4200-4205, 1973
- [12] Setlow JK, Setlow RB: Nature of the photoreactivable ultra-violet lesion in DNA. Nature 197:560-562, 1963
- [13] McDonell M, Simon MN, Studier FW: Analysis of restriction fragments of T7 DNA and determination of molecular weights by electrophoresis of neutral and alkaline gels. J. Molec. Biol. 110:119-143, 1977
- [14] Achey PM, Woodhead AD, Setlow RB: Photoreactivation of pyrimidine dimers in DNA from thyroid cells of the teleost, Poecilia formosa. Photochem. Photobiol. 29:305-310, 1979
- [15] Cook JS, Regan JD: Photoreactivation and photoreactivating enzyme activity in an order of mammals (Marsupilia).

Nature 223:1066-1067, 1969

- [16] Sutherland BM: Photoreactivating enzyme from human leukocytes. Nature 248:109-112, 1974
- [17] Harm H: Damage and repair in mammalian cells after ultraviolet and/or visible light treatment. Symposium on Biological effects and measurement of light sources, proceedings. Edited by DG Hazzard. Rockville, MD, pp 175-193, 1976. HEW publication (FDA) 77-8002
- [18] Sutherland JC, Sutherland BM: Human photoreactivating enzyme: Action spectrum and safelight conditions. Biophys. J. 15:435-440, 1975
- [19] Sutherland BM, Oliver R, Fuselier CO, Sutherland JC: Photoreactivation of pyrimidine dimers in the DNA of normal and xeroderma pigmentosum cells. Biochemistry 15:402-406, 1976
- [20] Wagner EK, Rice M, Sutherland BM: Photoreactivation of Herpes simplex virus in human fibroblasts. Nature 254:627-628, 1975
- [21] Henderson EE: Host cell reactivation of Epstein-Barr virus in normal and repair-defective leukocytes. Cancer Res. 38:3256-3263, 1978
- [22] Chiang T, Rupert CS: Action spectrum for photoreactivation of ultraviolet irradiated marsupial cells in tissue culture. Photochem. Photobiol. 30:525-528, 1979

- [23] Van der Leun JC, Stoop T: Photorecovery of ultraviolet Erythema. In The Biologic Effects of Ultraviolet Radiation. Edited by F Urbach. Pergamon Press, Oxford, pp 251-254, 1969
- [24] Willis I, Kligman A, Epstein J: Effects of long ultraviolet rays on human skin: Photoprotective or photoaugmentative. J. Invest. Dermat. 59:416- , 1973
- [25] Ying CY, Parrish JA, Pathak MA: Additive erythemogenic effects of middle - (280-320 nm) and long - (320-400 nm) wave ultraviolet light. J. Invest. Dermat. 63: 273- , 1974
- [26] Van Weelden H: Photoreactivation in human skin. Lasers in Photomedicine and Phototherapy. Europhysics Conference (in Conference Digest), p 25, 1979
- [27] Cooke A, Johnson BE: Dose response, wavelength dependence and rate of excision of ultraviolet radiation - induced pyrimidine dimers in mouse skin DNA. Biochem. Biophys. Acta 517:24-30, 1978
- [28] Pathak MA, Kramer DM, Gungerich U: Formation of thymine dimers in mammalian skin by ultraviolet radiation in vivo. Photochem. Photobiol. 15:177-185, 1972
- [29] Bowden GT, Trasko JE, Shapas BG, Boutwell RK: Excision of pyrimidine dimers from epidermal DNA and non-semi-conservative epidermal DNA synthesis following UV

irradiation of mouse skin. Cancer Res. 35:3599-3607,
1975

- [30] Strickland PT: Pyrimidine dimer formation in epidermal DNA and oncogenesis in rat skin exposed to ultraviolet radiation. (Ph.D. thesis) New York University, 1978
- [31] Ley RD, Sedita BA, Grube DD: Absence of photoreactivation of pyrimidine dimers in the epidermis of hairless mice following exposures to ultraviolet light. Photochem. Photobiol. 27:483-485, 1978
- [32] Regan JD, Setlow RB: Repair of chemical damage to human DNA. In Chemical Mutagens. Principles and Methods for their Detection. Edited by A Hollaender. Plenum, NY, pp 151-170, 1973
- [33] Ananthaswamy HN, Fisher MS, Kripke ML: Photoreactivation of UV-induced pyrimidine dimers in neonatal BALB/C mouse skin. 8th Ann. Meeting, American Soc. for Photobiology, Colorado Springs, p 67, 1980
- [34] Epstein JH, Fukuyama K, Reed WB, Epstein WL: Defect in DNA synthesis in skin of patients with xeroderma pigmentosum demonstrated in vivo. Science 168:1477-1478, 1970
- [35] Sutherland BM, Kochevar I, Harber L: Pyrimidine dimer formation and repair in human skin. Cancer Research, in press, 1980
- [36] Ahmed FE, Setlow RB: DNA repair in xeroderma pigmentosum

cells treated with combinations of ultraviolet
radiation and N-acetoxy-2-acetylamino fluorene.

Cancer Res. 39:471-479, 1979

FIGURE LEGENDS

Figure 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.

FIGURE LEGENDS

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

FIGURE LEGENDS

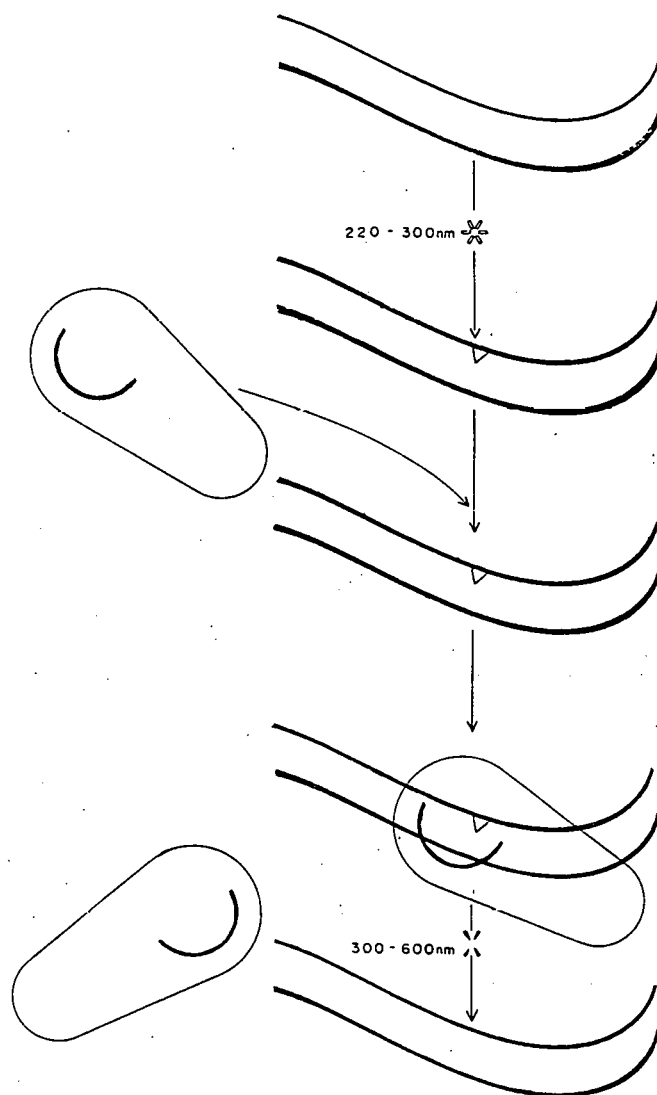
Figure 3. Action spectra for photoreactivation by isolated human photoreactivating enzyme (lower curve) and for cellular dimer photoreactivation by human cells in culture. Reprinted with permission from Sutherland, B. M., Oliver, R., Fuselier, C. O. and Sutherland, J. C. *Biochemistry* 15, pp. 402-405, 1976. Copyright 1976, American Chemical Society.

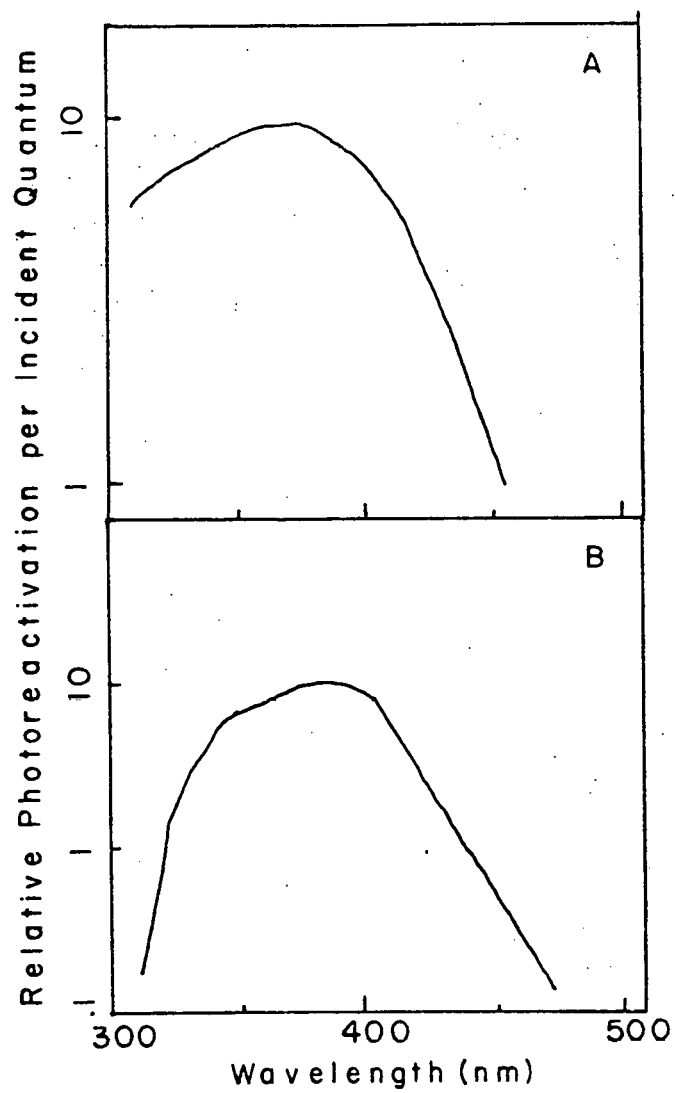
FIGURE LEGENDS

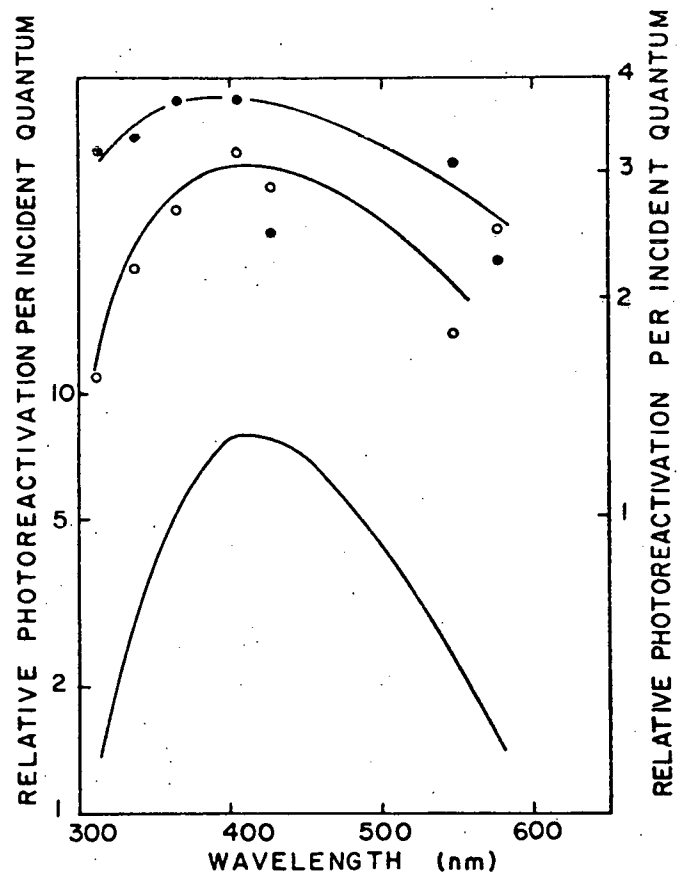
Figure 4. Pyrimidine dimer measurement by alkaline agarose gel electrophoresis. Cells are untreated (left column) or UV-irradiated (right column). The cellular DNA is extracted, and treated with an endonuclease which makes a single-strand nick adjacent to each dimer. The DNA is denatured in alkali and 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.

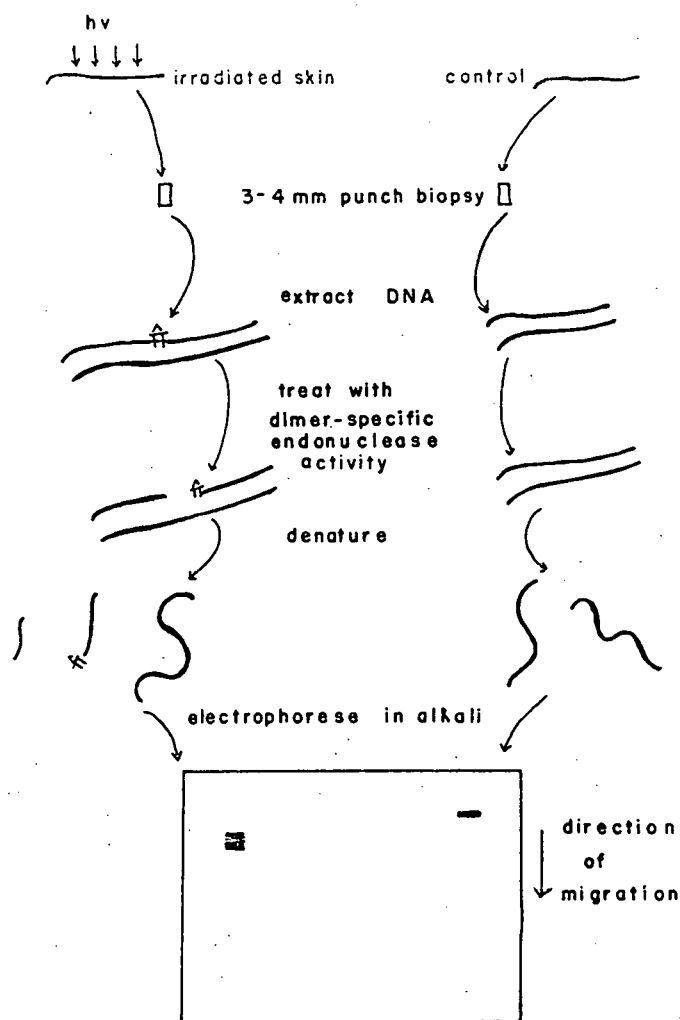
FIGURE LEGENDS

Figure 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 post-irradiation in the dark (panel C) or after 20 min exposure to visible light (panel D). Reprinted with permission from Sutherland, B. M., Kochevar, I. and Harber, L. Cancer Res., in press, 1980.









PHOTOREACTIVATION IN BACTERIA AND IN SKIN

Betsy M. Sutherland, Ph.D.

Department of Biology

Brookhaven National Laboratory

Upton, NY 11973 USA

Send proofs and reprint requests to author and address above.

Running head: Photoreactivation