tor prepared from rat liver cytosol in the presence of 20 m*M* Na_2MoO_4 (*15*). 20-Dihydroprednisolonic acid failed to stabilize lysosomal membranes or replace labeled dexamethasone bound to the receptor. In addition, the steroid acid esters showed no inhibitory activity on rat skin collagen synthesis and did not cause skin atrophy when administered subcutaneously (*16*).

These data substantiate the hypothesis that both steroid keto acid and hydroxy acid esters which retain the intact ring structures of potent corticosteroids possess anti-inflammatory activity but upon entry into the circulatory system from the administration site are hydrolyzed to inactive steroid acids. Thus, these acid ester derivatives have minimal adverse systemic effects. The fact that anti-inflammatory activity of the steroid acid esters was not accompanied by PA suppression after local and systemic administration suggests that the anti-inflammatory activity of corticosteroids may be separate from the PA suppressive activity.

Typically, the C-20 carbonyl function has been considered essential for anti-inflammatory activity. No glucocorticoid currently in clinical use has a reduced keto group, that is, a hydroxy group, at the C-20 position as is present in methyl 20-dihydroprednisolonate. It is therefore significant that the corresponding C-20 hydroxy compound is not only an active local anti-inflammatory agent but also is as potent as the C-20 keto compound.

We suggest that a new term, antedrug, in contrast to the term prodrug coined by Albert (*17*), be applied to active compounds formed by chemical modification of an active parent compound, when the new compound is rapidly metabolized to an inactive compound upon entry into the circulation from the tissue to which it was applied. Thus the antedrug acts only locally. The steroid-21-oate esters discussed in this report serve to exemplify this concept.

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References and Notes

1. T. L. Popper and S. S. Watnick, in *Medicinal Chemistry*, vol. 13, part 1, R. A. Scherrer and M. W. Whitehouse, Eds. (Academic Press, New York, 1974), pp. 247–256.
2. A. W. McKenzie and R. B. Stoughton, *Arch. Dermatol.* **86**, 608 (1962); E. F. Collins, J. Ashenbrenner, M. Nakakama, *Steroids* **20**, 543 (1972); W. M. Heroman, D. E. Bybee, M. Cardin, J. Bass, K. E. Johnsonbough, *J. Pediatr.* **96**, 500 (1980).
3. J. E. Nilsson and L. J. Gip, *Acta Dermatol. Kyoto Engl. Ed.* **59**, 245 (1979); D. D. Munro, *Br. J. Dermatol.* **94**, 67 (1976); N. L. Gottlieb and N. S. Pennys, *J. Am. Med. Assoc.* **243**, 1260 (1981); I. Engstroem and E. Oberger, *Scand. J. Respir. Dis.* **101**, 189 (1977); A. Leonard, A. Henrick, L. M. Silverman, *Am. J. Ophthalmol.* **87**, 210 (1979).
4. For examples of steroids with a modified 17-ketol side chain and retaining the anti-inflammatory activity of the parent compounds, see (*5*); M. J. Bussee *et al.*, *Br. J. Dermatol.* **81**, 103 (1969); C. A. Schlagel, *J. Pharm. Sci.* **54**, 335 (1965).
4a. E. Laurent, E. Gerhard, R. Weichert, *Angew. Chem. Int. Ed. Engl.* **14**, 65 (1975).
5. M. R. I. Soliman, Z. M. Nathoo, A. S. Heiman, E. Cook, H. J. Lee, in *Progress in Research and Clinical Applications of Corticosteroids*, H. J. Lee and T. J. Fitzgerald, Eds. (Heyden, Philadelphia, 1981), pp. 253–267.
6. See V. W. Mutzel, *Drug Res.* **27**, 2230 (1977), for metabolism of steroidal acid esters, and M. R. I. Soliman and H. J. Lee, *Res. Commun. Chem. Pathol. Pharmacol.* **33**, 357 (1981), for inactivity of an acidic metabolite.
7. Methyl 11β,17-dihydroxy-3,20-dioxo-1,4-pregnadiene-21-oate was prepared by diazomethane methylation of the corresponding acid which was synthesized from 20-dehydroprednisolone by the method of C. Monder [*Steroids* **18**, 187 (1971)]. The steroid acid ester was purified by chromatography on a silica gel 60 column with a mixture of hexane, dichloroethane, and acetone, and crystallized from hexane and acetone. The structure was confirmed by elemental analysis and nuclear magnetic resonance.
8. Methyl 11β,17-20ξ-trihydroxy-3-oxo-1,4-pregnadiene-21-oate was prepared according to the method of M. L. Lewbart and V. R. Mattox [*J. Org. Chem.* **28**, 1779 (1963)], and purified as described in (*6*). Hydrolysis of the ester with NaOH yielded 20-dihydroprednisolonic acid.
9. R. Meier, W. Schuler, P. Desaulles, *Experientia* **6**, 469 (1950).
10. S. A. Berson and R. S. Yalow, *J. Clin. Invest.* **47**, 2725 (1968). The ACTH kit was purchased from Amersham Corp., Arlington Heights, Illinois.
11. J. Vernikos-Danellis, E. Anderson, L. Trigg, *Endocrinology* **79**, 624 (1966).
12. S. Seifer and S. Dayton, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **8**, 249 (1949).
13. H. Laurent, E. Gerhard, R. Wiechert, *J. Steroid Biochem.* **6**, 185 (1975).
14. A. S. Heiman and H. J. Lee, *Steroids* **38** (No. 4), 365 (1981).
15. H. J. Lee, H. L. Bradlow, M. C. Moran, M. K. Sherman, *J. Steroid Biochem.* **14**, 1325 (1981).
16. K. R. Cutroneo, T. DePetrillo, H. J. Lee, in preparation.
17. A. Albert, *Nature (London)* **182**, 421 (1958).
18. Supported by NIH grants AM 21627 and RR 08111. We thank L. Bradlow, T. Fitzgerald, K. Cutroneo, and C. Monder for valuable comments.

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Genetic Damage in *Escherichia coli* K12 AB2480 by Broad-Spectrum Near-Ultraviolet Radiation

Abstract. *Irradiation with either broad-spectrum near-ultraviolet [fluorescent BLB (black light blue)] or monochromatic wavelengths in the near-ultraviolet range (320 to 400 nanometers) can cause specific damage to DNA as shown in experiments with* Escherichia coli *K12 AB2480 at the stationary phase of growth.*

Renewed interest in the biological effects of exposure to solar ultraviolet (UV) radiation (295 to 400 nm) has revealed uncertainties in our knowledge of the kind of damage produced in cells, plants, and animals (including humans) to wavelengths in the 295- to 320-nm (mid-UV) range and the 320- to 400-nm (near-UV) range (*1–3*). Three major questions remain. (i) Do wavelengths longer than 320 nm produce significant direct or indirect effects in genetic material? (ii) Do the biological effects follow the absorption spectrum of DNA at wavelengths greater than 310 nm? (iii) Does the sum of the effects of single wavelengths equal the effect of broad-spectrum near-UV radiation, such as solar UV, over the same wavelength range? Because of light scatter in solutions of DNA, it has not been possible to measure accurately the true absorption of DNA at wavelengths longer than 320 nm, but progress has been made (*4*). Other evidence suggests that many of the biological effects observed at wavelengths longer than 320 nm occur through indirect mechanisms such as photodynamic action, which is oxygen-enhanced, or triplet sensitization, which is not enhanced by oxygen (*5–8*). The answers to these questions are important in the practice of dentistry (*9*) and dermatology (*3*), in the assessment of biological consequences of the depletion of stratospheric ozone (*1*), and in medical and recreational uses of mid-UV and near-UV wavelengths (*3*, *9*).

We have compared the production of cyclobutylpyrimidine dimers in *Escherichia coli* DNA by broad-spectrum near-UV radiation with that by monochromatic wavelengths within the same wavelength band, using the specificity of enzymatic photoreaction (PR) (*10*) as the basis of a biological assay of the pyrimidine dimers produced.

The radiation-sensitive variant *E. coli* K12 AB2480 (*recA uvrA*) (Fig. 1B) was incubated at 37°C for 48 hours on the surface of nutrient agar (Difco). Cells in the stationary phase of growth were used for the biological assay of radiation sensitivity and photoreactivability. The cells were suspended at approximately 10^8 cells per milliliter and centrifuged twice before resuspension in M9 buffer (*p*H 7.0) at the same concentration (*11*). Irradiation was carried out at high fluence rates and at 0°C to reduce the possibility of concomitant photoreactivation, since fluorescent BLB (black light blue) radiation is within the effective wavelength range of enzymatic PR. The BLB light

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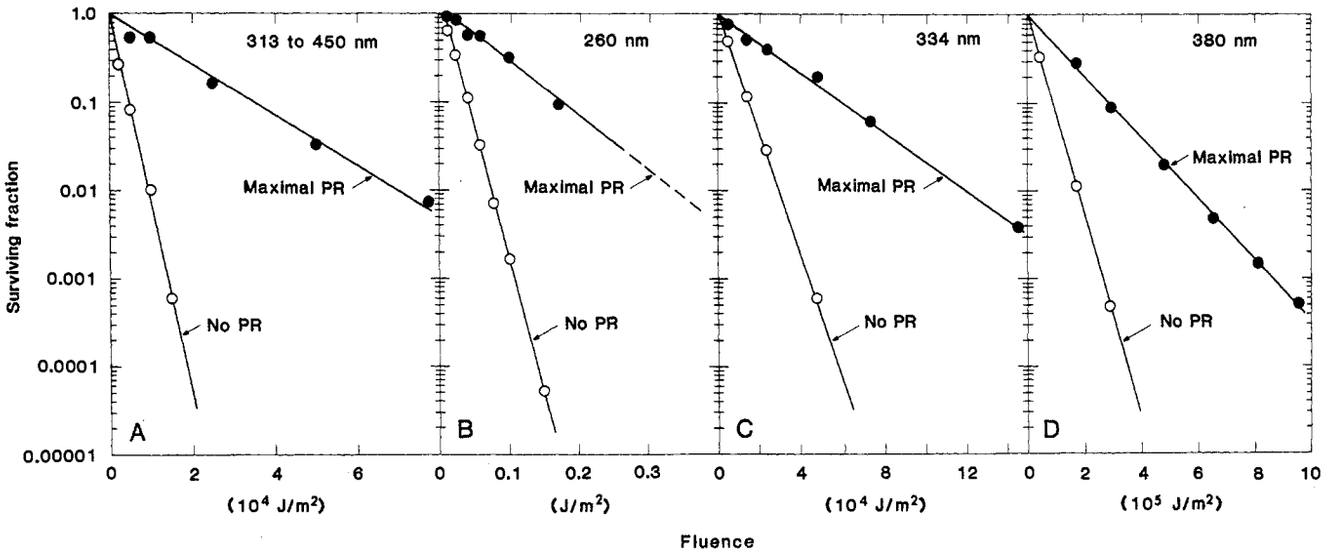

Fig. 1. Cell killing of *E. coli* K12 AB2480 in stationary phase in the presence (closed symbols) and absence (open symbols) of maximal PR (400-nm light at 20 W/m^2 for 30 minutes at 25°C, fluence of 3.6 × 10^4 J/m^2) after exposure to lethal fluences of radiation. (A) Lethality by fluorescent BLB radiation with a PR ratio of 7.1:1; (B) lethality by 260-nm radiation with a PR ratio of 4.1:1; (C) lethality by 334-nm radiation with a PR ratio of 4.1:1; and (D) lethality by 380-nm radiation with a PR ratio of 3.3:1. Fluence rates for lethality were 65 W/m^2 with BLB radiation, 0.0035 W/m^2 at 260 nm, 400 W/m^2 at 334 nm, and 350 W/m^2 at 380 nm. Typical single experiments are shown; each protocol was repeated at least two times.

source, especially designed to yield a high fluence rate, was an array of twelve 4-W bulbs arranged around a 5-cm-square chamber with three bulbs on a side. The unit was operated at 135 V, which increased the radiant emission 35 percent above that at 115 V. The Pyrex irradiation vessel was cylindrical, with an inside diameter of 1 cm. The vessel was surrounded by a cylindrical Pyrex jacket through which a thermostatically controlled mixture of 15 percent ethanol in distilled water was circulated to control the temperature of the sample during irradiation. Single wavelengths in the near-UV range were obtained from a 2.5 kW mercury-xenon monochromator. The radiation sources and the stray-light filters used for obtaining monochromatic radiation have been described (*7*). Photoreactivation was conducted at a low fluence (3.6 × 10^4 J/m^2) at 25°C (*12, 13*). The beam from a quartz iodine lamp mounted in a modified slide projector was filtered by a solution of $CuSO_4$, $CoSO_4$, and an absorption filter (Optics Technology LP400), yielding an emission peak at approximately 400 nm with little energy (< 5 percent) emitted below 380 nm. Fluence rates were measured with a calibrated Kettering radiometer (model 65, Yellow Springs Instrument).

Photoreactivation at 25°C after lethal fluences of BLB radiation at 0°C is shown in Fig. 1A, and PR after irradiation at representative monochromatic wavelengths is indicated in Fig. 1, B to D. The PR fluence-reduction ratio (ratio of the slope in the absence of PR with that in its presence) was 7.1:1 after broad-spectrum BLB radiation (spectral range, 313 to 405 nm). In contrast, the PR ratios after lethal irradiation with the monochromatic wavelengths were all significantly lower—4.1:1 with 260-nm radiation, a far-UV wavelength near the widely used wavelength of 254 nm (Fig. 1B); 4.1:1 with 334-nm radiation (Fig. 1C); and 3.1:1 with 380-nm radiation (Fig. 1D). The monochromatic wavelengths 334 nm and 380 nm lie well within the fluorescent BLB emission spectral range. In addition, the cells were more sensitive to killing by BLB radiation than by 334 nm (3.5-fold), 380 nm (20-fold), or any wavelength tested above 320 nm (*2, 6, 7*).

Different kinds of data clearly demonstrate that the photoreactivation demonstrated under these four sets of conditions is of the enzymatic type (*2, 10, 12*). Furthermore, Tyrrell (*14*), using the method of Carrier and Setlow (*15*), chemically identified cyclobutylpyrimidine dimers produced by monochromatic 365-nm radiation in cellular DNA. These dimers were readily monomerized by yeast PR enzyme with light and by direct exposure to monochromatic 235-nm radiation without the PR enzyme. In addition, concomitant PR can be demonstrated after exposure to monochromatic 365-nm radiation (*12*) and with broad-spectrum BLB radiation by comparing results at 25°C and 0°C (data not shown). Therefore, on the basis of the specificity of enzymatic PR (*10*), we conclude that both monochromatic radiation in the wavelength range 334 to 380 nm and broadband near-UV BLB radiation (313 to 405 nm) can produce cyclobutylpyrimidine dimers in yields great enough to account for many of the biological effects observed. However, wild-type and *uvrA* strains have such a great capacity to repair cyclobutyl dimers produced by near-UV radiation that their contribution to cell killing is below detection (*13*). In contrast, near-UV–induced dimers can be demonstrated to be mutagenic (*2, 16*). Various additional DNA lesions have been shown to be produced in cells by near-UV radiation. These lesions include DNA single-strand breaks (*5, 17*), alkali-labile bonds (*17*), and pyrimidine glycols (*18*). Damage to various DNA repair systems by near-UV radiation may modify the effects of DNA lesions (*19, 20*). Furthermore, effects in human tissue have been reported by Zigman *et al.* (*21*), who have shown that damage to various parts of the human eye can occur from exposure to different near-UV sources.

Genetic damage produced by near-UV radiation is not limited to bacteria: endonuclease sites (*22, 23*), single-strand breaks (*23*), and sister chromatid exchanges (*23*) have been produced by a near-UV wavelength (365 nm) in mouse and hamster cells. There is little doubt that similar DNA lesions are produced in human cells (*24*). These results have special significance because of the recent increased use of tanning booths and tanning beds employing both mid-UV and near-UV wavelengths (*3, 25*).

Caution should be exercised in the presence of near-UV radiation, including the near-UV component of sunlight (*2, 3, 22, 24*). If a functional PR enzyme is

present in human tissue, as has been suggested by Sutherland (*26*), concomitant PR during near-UV irradiation, interacting with efficient dark DNA repair systems, should reduce but not eliminate DNA damage from solar UV radiation. However, if an effective PR enzyme is not present in human skin or the human eye, stringent precautions are appropriate when the possibility exists of exposure to moderate to high fluences of near-UV radiation.

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References and Notes

1. R. B. Setlow, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3363 (1974).
2. R. B. Webb, in *Photochemical and Photobiological Reviews*, K. C. Smith, Ed. (Plenum, New York, 1977), pp. 169–268.
3. J. A. Parrish, R. R. Anderson, F. Urbach, D. Pitts, *UV-A: Biological Effects of Ultraviolet Radiation with Emphasis on Human Responses to Longwave Ultraviolet* (Plenum, New York, 1978).
4. J. C. Sutherland and K. P. Griffin, *Radiat. Res.* **86**, 399 (1981).
5. R. M. Tyrrell, R. D. Ley, R. B. Webb, *Photochem. Photobiol.* **20**, 395 (1974).
6. R. B. Webb and M. S. Brown, *Int. J. Radiat. Biol.* **36**, 671 (1979).
7. ———, *Photochem. Photobiol.* **29**, 407 (1979).
8. R. M. Tyrrell, *ibid.* **23**, 13 (1976).
9. D. C. Birdsell, P. J. Bannon, R. B. Webb, *J. Am. Dent. Assoc.* **94**, 311 (1977).
10. J. K. Setlow, in *Comprehensive Biochemistry, Photobiology, Ionizing Radiation*, M. Florkin and E. H. Stotz, Eds. (Elsevier, New York, 1967), p. 157.
11. E. H. Anderson, *Proc, Natl. Acad. Sci. U.S.A.* **32**, 120 (1946).
12. M. S. Brown and R. B. Webb, *Mutat. Res.* **15**, 348 (1972).
13. R. B. Webb, M. S. Brown, R. M. Tyrrell, *ibid.* **37**, 163 (1976).
14. R. M. Tyrrell, *Photochem. Photobiol.* **17**, 69 (1973).
15. W. L. Carrier and R. B. Setlow, *Methods Enzymol.* **21**, 230 (1971).
16. R. B. Webb, *J. Bacteriol.* **133**, 860 (1978).
17. R. D. Ley, A. Sedita, E. Boye, *Photochem. Photobiol.* **27**, 323 (1978).
18. P. Cerutti, cited in R. B. Webb, in *Photochemical and Photobiological Reviews*, K. C. Smith, Ed. (Plenum, New York, 1977), pp. 169–268.
19. R. M. Tyrrell and R. B. Webb, *Mutat. Res.* **19**, 361 (1973).
20. R. B. Webb and M. S. Brown, *Radiat. Res.* **80**, 82 (1979).
21. S. Zigman, J. Schultz, T. Yulo, G. Gross, *Exp. Eye Res.* **15**, 201 (1973).
22. R. H. Rothman and R. B. Setlow, *Photochem. Photobiol.* **29**, 57 (1979).
23. T. Matsushita, Argonne National Laboratory, personal communication.
24. G. J. Kantor, J. C. Sutherland, R. B. Setlow, *Photochem. Photobiol.* **31**, 459 (1980).
25. D. S. Nachtwey and R. D. Rundel, *Science* **211**, 405 (1981).
26. B. Sutherland, *Basic Life Sci.* **5A**, 197 (1975).
27. Supported by the U.S. Department of Energy under contract No. W-31-109-ENG-38. We acknowledge significant discussions with Dr. M. J. Peak.

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Calcium-Bearing Objects Elicit Shell Selection Behavior in a Hermit Crab

Abstract. *Hermit crabs explore empty gastropod shells by touching, rolling, and probing them before choosing one for a home. This component of shell selection behavior was examined in* Pagurus hirsutiusculus hirsutiusculus *(Dana) with binary choice tests between natural shells and accurate replicas of the shells with different chemical compositions. The results show that calcium emanating from the surface of shells is responsible for the behavior. Sensitivity to calcium may be a factor that enables the hermit crab to locate partially buried shells and discriminate empty shells from ones housing living gastropods or from small pebbles.*

Many hermit crab species carefully select the gastropod shells they inhabit (*1–4*) and exhibit a complex behavioral sequence before accepting a shell (*2, 5*). Briefly, the selection process begins with the hermit crab grasping a shell with its walking legs and running its opened chelae over the surface. It then rolls the shell over between its appendages until it finds the aperture and probes the opening with the chelae. If the hermit crab lacks a shell, aperture probing is always followed by rapid insertion of the crab's abdomen into the shell (Fig. 1).

The specific features of shells that the crab examines and how each independently affects the choice of a shell was not known (*6, 7*). To study this, replicas of natural shells, including their fine textural features, were constructed. Original shells served as a standard for artificial replicas of different compositions. Independent variation of shell parameters allowed assessment of the contribution of each to shell selection (*8*).

Using this technique, I found that calcium promotes the exploration of shells and further examination of other features by *Pagurus hirsutiusculus hirsutiusculus* (Dana). Calcium-bearing objects such as pieces of minerals also elicited shell-like exploration behavior. Although, for other species visual characteristics promote investigation as well (*2*), shells lacking calcium were rarely investigated by *P. hirsutiusculus* (*9*), suggesting that initial recognition and examination of a shell is primarily dependent on a chemical cue (*10*).

Table 1. Results obtained from binary choice tests with the hermit crab *P. hirsutiusculus*.

Condition	Number of animals choosing		Number of animals
	Natural shell	Replica shell	
Seawater	0	26	26
Seawater saturated with $CaSO_4$	13*	9*	22
No visual information, seawater	0	8	8

*Not significantly different by the chi-square test.

The intertidal hermit crab *P. hirsutiusculus* prefers gastropod mollusc shells of the genus *Nucella* (*11*). Both male and female crabs with cephalothorax lengths in the range 0.7 to 1.6 cm were used. Hermit crabs were collected off Heceta Head, Oregon, from March through September and maintained in aquariums 1 m in diameter in a 12:12 hour, light:dark cycle. Natural seawater was used with a *p*H range of 7.8 to 8.0, at a constant temperature of 15°C.

Replicas of natural shells were made from inside and outside molds between which reagent grade, plaster of Paris ($CaSO_4$ $1/2H_2O$, J. T. Baker Company) and water were poured (*12*). Natural shells were boiled, and both replica and natural shells were handled with disposable gloves (*13*) to control for additional chemical cues. Shell selection was examined by binary choice tests between a natural and replica shell or a pair of replicas of the gastropod shell *Nucella emarginata* or *N. canaliculata*. Crabs were removed from the shells in which they were found and placed in an opaque, round test chamber with an exit to a larger container. Two shells (choices), side by side with their apertures down, were placed directly outside the exit. Animals were tested only once. Shell positions were alternated between trials to control for side preferences. Except for animals tested in complete darkness, crabs were scored only if they contacted both shells.

To test the effects on behavior of particular minerals, a sample was placed in the test chamber at a fixed position just outside the crab's refuge (a few rocks). Animals were given a maximum of five contacts with only one sample and scored if full exploratory behavior was observed (*14*).

In tests of selection between plaster replica shells and natural shells, 26 of 26 hermit crabs preferred replica shells. Hermit crabs that were in contact with both shells explored the surface of the plaster replica shells first; further exploration was followed by acceptance of the plaster shells.

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