(*10*). Since no mononuclear inflammatory cells have been observed in this lesion, it has been proposed that resident fibroblasts are responsible for the matrix destruction. It is conceivable that catabolic factors of the variety secreted from porcine mitral and aortic valves mediate such tissue injury.

The present observations imply a possible role for such factors during development of degenerative disease; however, their mode of action implies that they might function in the normal turnover of extracellular macromolecules as well. Tissue catabolins may also be active during wound healing. Since human and porcine synovium fail to produce catabolin in the presence of hydrocortisone (*11*), the poor wound healing that frequently attends corticosteroid therapy may well reflect an inhibition of turnover and repair processes that require these factors. Therefore, the hypothesis that catabolins may have a more general role in cell-tissue interactions may be pertinent not only to our understanding of the mechanisms that modify tissue integrity but also to the pharmacological control of tissue damage in catabolic diseases.

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## Anti-Inflammatory Steroids Without Pituitary-Adrenal Suppression

Abstract. *When two new steroids, methyl prednisolonate and methyl 20-dihydroprednisolonate, were applied locally their anti-inflammatory activities were nearly equivalent to those of the parent compound prednisolone in the cotton pellet granuloma bioassay. However, when these two derivatives were administered systemically, their anti-inflammatory activities were weaker than those of the parent compound. Furthermore, unlike the parent compound, these new anti-inflammatory steroids did not suppress pituitary-adrenal function or cause liver glycogen depletion in rats.*

Although the beneficial effects of natural and semisynthetic corticosteroids in the treatment of inflammatory and allergic conditions have been appreciated for over 30 years, complications arising from steroid therapy have imposed limitations on the clinical use of this class of drugs (*1*). The shortcomings are largely inherent in the nature of corticosteroids themselves; not only do glucocorticosteroids possess multiple biological activities, but the structural requirements for various activities appear to be overlapping and inseparable. If the actions of corticosteroids could be localized, many of the complications could be eliminated. Although methods for the local administration of steroids have been devised (*2*), complications associated with local steroid treatment for psoriatic, rheumatologic, eczematous, asthmatic, and ophthalmic patients have been reported (*3*). This situation calls for new approaches in developing anti-inflammatory steroids that are devoid of toxicities.

In developing a new concept, we were guided by several considerations: (i) corticosteroid pharmacotherapy appears to offer an abundance of agents, but no truly safe drug; (ii) systemic effects of steroids are unnecessary complications which accompany treatment of many inflammatory conditions; (iii) an intact ketol side chain is not an absolute requirement for the anti-inflammatory activity of corticosteroids (*4*, *4a*, *5*); and (iv) steroid acid esters with intact ring structures corresponding to the known potent glucocorticoids retain anti-inflammatory activity but upon entry into the circulatory system from the site of administration are hydrolyzed to steroid acids that are inactive and readily excreted (*6*).

We now report that ester derivatives of steroid-21-oic acids, applied locally, possess anti-inflammatory activity equivalent to the parent compound but do not suppress adrenal function or liver glycogen content in rats.

The anti-inflammatory activities of methyl prednisolonate (*7*), methyl 20-dihydroprednisolonate, and 20-dihydroprednisolonic acid (*8*) were evaluated in the cotton pellet granuloma bioassay in rats (*9*). Thymolytic, liver glycogen depository, and pituitary-adrenal (PA) suppressive effects were monitored. When the rats were under mild anesthesia we implanted two cotton pellets (35 ± 1 mg each) subcutaneously, one in each axilla. The local effects of the steroids on granuloma formation were determined by injection of the compound into the cotton pellet before implantation; the systemic effects were evaluated by giving daily intramuscular injections of the compounds after pellet implantation. Seven days after implantation the rats were killed and granuloma, adrenal, thymic, and body weights were measured. Blood samples were analyzed for adrenocorticotropin (ACTH) (*10*) and corticosterone (*11*) and livers were analyzed for glycogen content (*12*).

Prednisolone caused a significant decrease in all the values measured in control rats. In contrast, the new steroids methyl prednisolonate and methyl 20-dihydroprednisolonate, when they were administered locally, selectively suppressed the weights of granulomas and thymus glands but did not alter adrenal weights, plasma ACTH, plasma corticosterone, or liver glycogen (Fig. 1A). In this study high doses of steroids were administered deliberately in order to detect any possible systemic toxicities. At the dose level of 2.5 mg per pellet, methyl prednisolonate and methyl 20-dihydroprednisolonate decreased granuloma formation by 56.9 percent and 58.6 percent, respectively. These values are comparable to the 58.3 percent granuloma inhibition obtained with the same dose of the parent compound prednisolone, and suggest that some degree of freedom is available for modifying the ketol side chain of corticosteroids without losing anti-inflammatory activity. This is supported by the anti-inflammatory activity reported for the fluocortolonate esters (*4a*, *13*). However, with doses of 5 mg per pellet, prednisolone exhibited a higher inhibitory effect on granuloma formation (70.29 percent) than either methyl prednisolonate (55.6


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percent) or methyl 20-dihydroprednisolonate (36.3 percent).

The anti-inflammatory activity of the steroid acid esters, unlike prednisolone, appears to reach a plateau at the dose of 2.5 mg per pellet. The significant thymolytic effects observed with the steroid acid esters at the high local dose when applied to the axillae compared to the lack of thymolytic effect with systemic administration suggests that some diffusion of the steroids occurs from the application sites into adjacent thymus tissue. However, despite this diffusion, the local anti-inflammatory activity of the steroid acid esters was not accompanied by decreases in adrenal weight or in plasma ACTH and corticosterone. A metabolite of the esters, which showed no anti-inflammatory activity was, as expected, devoid of thymolytic, glycogen depletion, and adrenal suppressive effects.

Although the steroid acid esters retained effective anti-inflammatory activity when administered intramuscularly for 7 days, their activity was weaker than that of prednisolone (Fig. 1B). At doses of 2.5 mg/kg, methyl prednisolonate and methyl 20-dihydroprednisolonate decreased granuloma formation by 16.07 and 15.2 percent, respectively, whereas prednisolone suppression was 42.6 percent. A similar trend in the relative potency of the steroids was observed at doses of 5 mg/kg. In contrast to the parent compound, methyl prednisolonate and methyl 20-dihydroprednisolonate exhibited no thymolytic or PA suppressive activities.

At doses of 5 mg/kg, methyl prednisolonate did not deplete liver glycogen at all, whereas methyl 20-dihydroprednisolonate slightly decreased liver glycogen content. At a dose of 10 mg/kg administered intramuscularly for 7 days, methyl 20-dihydroprednisolonate suppressed granuloma formation to the same degree as prednisolone administered at 2.5 mg/kg for the same period. However, the equipotent dose of methyl 20-dihydroprednisolonate did not suppress plasma ACTH or plasma corticosterone, although the parent compound did decrease their concentration (*5*).

We have reported recently that this new class of steroids inhibits the release of marker enzymes from rat liver lysosomes in vivo and in vitro (*14*), and replaces [$^3$H]dexamethasone from recep-

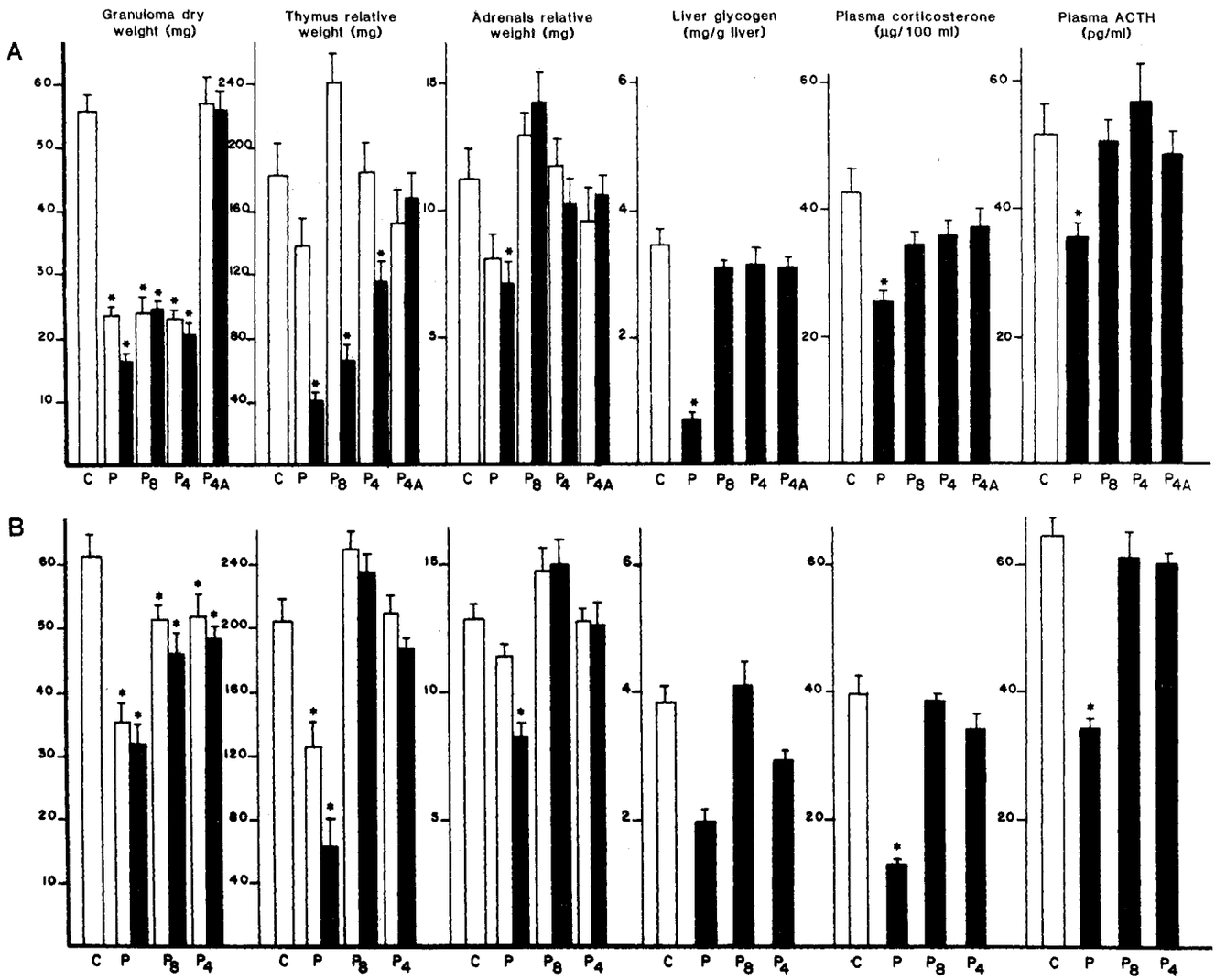


Fig. 1. The effects of prednisolone and its derivatives applied (A) locally and (B) systemically on granuloma formation, relative thymus and adrenal weights, liver glycogen, and plasma corticosterone and ACTH concentrations in male rats. The values presented are the means ± standard error of six animals. Relative thymus and adrenal weights are expressed as milligrams per 100 g of body weight. Open bars are values obtained at (A) a dose level of 2.5 mg per cotton pellet or (B) 2.5 mg/kg. Closed bars are values obtained at a dose of (A) 5 mg per cotton pellet or (B) 5 mg/kg. Symbols: *C*, control; *P*, prednisolone; $P_8$, methyl prednisolonate; $P_4$, methyl 20-dihydroprednisolonate; $P_{4A}$, 20-dihydroprednisolonic acid; *, significantly different from control values ($P < .05$).

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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## 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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