DEGRADATION OF HYDROCORTISONE BY *PSEUDOMONAS TESTOSTERONI*

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Corticosteroids are used extensively in topical medication. Clinicians have asked whether resident or transient skin microflora may decompose applied steroids and reduce their efficacy. Equally, steroids suffering covert contamination might also be transformed. This communication is concerned with the degradation of hydrocortisone by *Pseudomonas testosteroni* which is an obligate aerobic soil-borne organism capable of utilising many organic compounds as a sole source of carbon and energy (Marcus and Talalay, 1956) including C19 steroids based on the androstane nucleus (Talalay, 1957). This bacterium could occur as a contaminant in pharmaceutical products. A review of the literature coupled with an on-line computer search has failed to reveal any reports of the action of this organism on C21 corticosteroids.

10^6 Bacteria were incubated with hydrocortisone (100 µg/ml) in minimal medium (K_2HPO_4 7 g/l, KH_2PO_4 3 g/l, $MgSO_4.7H_2O$ 0.1 g/l, $(NH_4)_2SO_4$ 1 g/l; pH 7.2). Controls showed growth on citrate, a readily utilised carbon source, but no growth on methanol, the solvent vehicle used for steroid addition. Viable counts determined by a spread plate technique, showed multiplication of *Ps. testosteroni* at the expense of hydrocortisone. This growth reached a peak of 1.3×10^9 c.f.u./ml after 288 hr, a level equivalent to that obtained on citrate and well above that of the methanol control. A diauxic growth curve was obtained with citrate and hydrocortisone, which indicates that the enzymes involved in utilisation of hydrocortisone were inducible and accounts for a long lag phase when hydrocortisone was the carbon source. Thus *Ps. testosteroni* can utilise this corticosteroid as a source of carbon and energy. The transformations involved in this process were then studied.

Organisms grown in citrate reached a peak logarithmic phase after incubation for 42 hr. At this point hydrocortisone was added and incubation was continued for a further 48 hr. The whole culture was then extracted with chloroform which was evaporated and the residue redissolved in ethanol. Controls were included to account for any natural decomposition of steroid in the absence of bacterial growth. Thin layer chromatography of the products and authentic standards was carried out on 0.25 mm layers of Silica Gel GF Type 60 using the solvent system dichloromethane, ether, methanol, water (77:15:8:1.2 v/v), the resulting chromatogram being visualised under short wave ultra-violet light. The residue gave R_f values corresponding to prednisolone (0.37), 11β-hydroxy-androsta-1,4 diene-3,20 dione (0.59), unused hydrocortisone (0.43) and an unidentified spot (0.68). The organism appears to have converted the starter steroid by Δ1 dehydrogenation to the 1,4-diene-3-keto system of prednisolone followed by cleavage of the dihydroxyacetone side chain on Ring D to produce 11β-hydroxy-androsta-1,4-diene-3,20 dione. A likely residue of this pathway is glycolaldehyde. A similar pathway has been found in other organisms (Martin 1977).

Further work using bacteria isolated from normal skin showed no decomposition of hydrocortisone, prednisolone or triamcinolone acetonide.

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